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(57) Abstract

The present invention provides a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.

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RECEPTOR BASED ANTAGONISTS AND METHODS OF MAKING AND USING

This application claims priority of U.S. Application No. 09/313,942, filed May 19, 1999, which claims priority of U.S. Provisional Application No. 60/101,858 filed September 25, 1998. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

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BACKGROUND OF THE INVENTION

Although discovered for varying biological activities, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and interleukin-6 (IL-6) comprise a defined family of cytokines (referred to 15 herein as the "CNTF family" of cytokines). These cytokines are grouped together because of their distant structural similarities [Bazan, J. Neuron 7: 197-208 (1991); Rose and Bruce, Proc. Natl. Acad. Sci. USA 88: 8641-8645 (1991)], and, perhaps more importantly, because they share " β " signaltransducing receptor components [Baumann, et al., J. Biol. Chem. 20 265:19853-19862 (1993); Davis, et al., Science 260: 1805-1808 (1993); Gearing et al., Science 255:1434-1437 (1992); Ip et al., Cell 69: 1121-1132 (1992); Stahl, et al., J. Biol. Chem. 268: 7628-7631 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Receptor activation by this family of cytokines results from either homo- or hetero-dimerization of these β components [Davis, et al. 25 Science 260: 1805-1808 (1993), Murakami, et al., Science 260: 1808-1810 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. IL-6 receptor activation requires homodimerization of gp130 [Murakami, et al. Science 260: 1808-1810 (1993), Hibi, et al., Cell 63: 1149-1157 (1990)], a protein initially identified as the IL-6 signal transducer [Hibi, et al., Cell 63: 1149-1157 (1990)]. 30 CNTF, LIF and OSM receptor activation results from heterodimerization between gp130 and a second gp130-related protein known as LIFR\$ [Davis,

et al., Science 260: 1805-1808 (1993)], that was initially identified by its ability to bind LIF [Gearing et al., EMBO J. 10: 2839-2848 (1991)].

In addition to the β components, some of these cytokines also require 5 specificity-determining "a" components that are more limited in their tissue distribution than the β components, and thus determine the cellular targets of the particular cytokines [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus, LIF and OSM are broadly acting factors that may only require the presence of gp130 and LIFRβ on responding cells, while CNTF requires 10 CNTFRα [Stahl and Yancopoulos, Cell 74: 587-590 (1993)] and IL-6 requires IL-6Rα [Kishimoto, et al., Science 258: 593-597 (1992)]. Both CNTFRα (Davis et al., Science 259:1736-1739 (1993) and IL-6Rα [Hibi, et al. Cell 63:1149-1157, Murakami, et al., Science 260:1808-1810 (1990); Taga, et al., Cell 58:573-581 (1989)] can function as soluble proteins, consistent with the notion that they do not interact with intracellular signaling molecules but 15 that they serve to help their ligands interact with the appropriate signal transducing β subunits [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

Additional evidence from other cytokine systems also supports the notion that dimerization provides a common mechanism by which all cytokine receptors initiate signal transduction. Growth hormone (GH) serves as perhaps the best example in this regard. Crystallographic studies have revealed that each GH molecule contains two distinct receptor binding sites, both of which are recognized by the same binding domain in the receptor, allowing a single molecule of GH to engage two receptor molecules [de Vos, et al., Science 255: 306-312 (1992)]. Dimerization occurs sequentially, with site 1 on the GH first binding to one receptor molecule, followed by the binding of site 2 to a second receptor molecule [Fuh, et al., Science 256: 1677-1680 (1992)]. Studies with the erythropoietin (EPO) receptor are also consistent with the importance of dimerization in receptor activation, as EPO receptors can be constitutively activated by a

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single amino acid change that introduces a cysteine residue and results in disulfide-linked homodimers [Watowich, et al., Proc. Natl. Acad. Sci. USA 89:2140-2144 (1992)].

5 In addition to homo- or hetero-dimerization of β subunits as the critical step for receptor activation, a second important feature is that formation of the final receptor complex by the CNTF family of cytokines occurs through a mechanism whereby the ligand successively binds to receptor components in an ordered manner [Davis, et al. Science 260:1805-1818 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus CNTF first 10 binds to CNTFRα, forming a complex which then binds gp130 to form an intermediate (called here the $\alpha\beta1$ intermediate) that is not signaling competent because it has only a single β component, before finally recruiting LIFR β to form a heterodimer of β components which then 15 initiates signal transduction. Although a similar intermediate containing IL-6 bound to IL-6Rα and a single molecule of gp130 has not been directly isolated, we have postulated that it does exist by analogy to its distant relative, CNTF, as well as the fact that the final active IL-6 receptor complex recruits two gp130 monomers. Altogether, these findings led to a 20 proposal for the structure of a generic cytokine receptor complex (Figure 1) in which each cytokine can have up to 3 receptor binding sites: a site that binds to an optional α specificity-determining component (α site), a site that binds to the first β signal-transducing component (β 1 site), and a site that binds to the second β signal-transducing component (β 2 site) [Stahl 25 and Yancopoulos, Cell 74: 587-590 (1993)]. These 3 sites are used in sequential fashion, with the last step in complex formation -- resulting in β component dimerization — critical for initiating signal transduction [Davis, et al. Science 260:1805-1818 (1993)]. Knowledge of the details of receptor activation and the existence of the non-functional β1 30 intermediate for CNTF has led to the finding that CNTF is a high affinity

antagonist for IL-6 under certain circumstances, and provides the strategic basis for designing ligand or receptor-based antagonists for the CNTF family of cytokines as detailed below.

Once cytokine binding induces receptor complex formation, the 5 dimerization of β components activates intracellular tyrosine kinase activity that results in phosphorylation of a wide variety of substrates [Ip, et al. Cell 69:121-1132 (1992)]. This activation of tyrosine kinase appears to be critical for downstream events since inhibitors that block the tyrosine phosphorylations also prevent later events such as gene inductions [Ip, et 10 al., Cell 69:121-1132 (1992); Nakajima and Wall, Mol. Cell. Biol. 11:1409-1418 (1991)]. Recently, we have demonstrated that a newly discovered family of non-receptor tyrosine kinases that includes Jak1, Jak2, and Tyk2 (referred to as the Jak/Tyk kinases) [Firmbach-Kraft, et al., Oncogene 15 5:1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11: 2057-2065 (1991] and that are involved in signal transduction with other cytokines [Argetsinger, et al., Cell 74:237-244 (1993); Silvennoinen, et al., Proc. Natl. Acad. Sci. USA 90:8429-8433 (1993); Velazquez, et al., Cell 70: 313-322 (1992); Witthuhn, et al., Cell 74:227-236 (1993)], preassociate with the cytoplasmic domains of the β subunits gp130 and LIFR β in the absence of ligand, and become tyrosine 20 phosphorylated and activated upon ligand addition [Stahl et al., Science 263:92-95 (1994)]. Therefore these kinases appear to be the most proximal step of intracellular signal transduction activated inside the cell as a result of ligand binding outside of the cell. Assay systems for screening collections of small molecules for specific agonist or antagonist activities 25 based on this system are described below.

The CNTF family of cytokines play important roles in a wide variety of physiological processes that provide potential therapeutic applications for both antagonists and agonists.

SUMMARY OF THE INVENTION

An object of the present invention is the production of cytokine antagonists that are useful in the treatment of cytokine-related diseases or disorders.

Another object of the invention is the use of the disclosed cytokine antagonists for the treatment of cytokine-related diseases or disorders. For example, an IL-6 antagonist described herein may be used for the treatment of osteoporosis, the primary and second effects of cancers, including multiple myeloma, or cachexia.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of cytokine receptors.

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Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of the cytokines.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of members of the CNTF family of cytokines.

Another object of the invention is the development of screening systems
useful for identifying small molecules that act as agonists or antagonists of
the CNTF family of cytokines.

BRIEF DESCRIPTION OF THE FIGURES

30 FIGURE 1: Ordered binding of receptor components in a model of a generic cytokine receptor. The model indicates that cytokines contain up to 3 receptor binding sites and interact with their receptor components by

binding first the optional α component, followed by binding to $\beta 1$, and then $\beta 2$. The β components for many cytokine receptors interact through membrane proximal regions (shaded boxes) with the Jak/Tyk family of cytoplasmic protein tyrosine kinases. Only upon dimerization of β components is signal transduction initiated, as schematized by the tyrosine phosphorylations (P) of the β components and the Jak/Tyk kinases.

- FIGURE 2: CNTF inhibits IL-6 responses in a PC12 cell line (called PC12D) that expresses IL6Rα, gp130, CNTFRα, but not LIFRβ. Serum-deprived PC12D cells were incubated + IL-6 (50 ng/mL) in the presence or absence of CNTF as indicated. Some plates also received soluble IL6Rα (1 mg/mL) or soluble CNTFRα (1 mg/mL) as indicated. Cell lysates were subjected to immunoprecipitation with anti-gp130 and immunoblotted with anti-phosphotyrosine. Tyrosine phosphorylation of gp130 is indicative of IL-6 induced activation of the IL-6 receptor system, which is blocked upon coaddition of CNTF.
- FIGURE 3: Scatchard analysis of iodinated CNTF binding on PC12D cells. PC12D cells were incubated with various concentrations of iodinated CNTF in the presence or absence of excess non-radioactive competitor to determine the specific binding. The figure shows a Scatchard plot of the amount of iodinated CNTF specifically bound, and gives data consistent with two binding sites with dissociation constants of 9 pM and 3.4 nM.
- FIGURE 4. The amino acid sequence of human gp130-Fc-His6. Amino acids 1 to 619 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of gp130-Fc-His6 has been italicized (amino acids 1 to 22). The Ser-Gly bridge is shown in bold type (amino acids 620, 621). Amino acids 662 to 853 are from the Fc domain of human IgG1 (Lewis, et

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al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 632 and 635) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. The hexahistine tag is shown in bold/italic type (amino acids 854 to 859). (•) shows the position of the STOP codon.

FIGURE 5: The amino acid sequence of human IL-6Rα-Fc. Key: Amino acids 1 to 358 are from human IL-6Ra (Yamasaki, et al., Science 241:825-828 (1988). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of IL- $6R\alpha$ -Fc has been italicized (amino acids 1 to 19). The Ala-Gly bridge is shown in bold type (amino acids 359, 360). Amino acids 361 to 592 are from the Fc domain of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 371 and 374) of the IgG hinge preceding the Fc that form the 15 inter-chain disulfide bridges that link two Fc domains. (•) shows the position of the STOP codon.

FIGURE 6: The CNTF/IL-6/IL-11 receptor system. The ordered formation of the hexameric signal transducing receptor complex is depicted schematically. The cytokine associates with the Ra component to form an obligatory cytokine \bullet R α complex (Kd is about 5 nM). This low affinity complex next associates with the first signal transducing component, marked $\beta 1$, to form a high affinity cytokine $\bullet R\alpha \bullet \beta 1$ complex (Kd is about 10 pM). In the case of IL-6R α , this component is gp130. This trimeric high affinity complex subsequently associates with another such complex. Formation of this complex results in signal transduction as it involves dimerization of two signal transducing components, marked \$1 and \$2 respectively (adapted from (Ward et al., J. Bio. Chem. 269:23286-23289 (1994); Stahl and Yancopoulos, J. Neurobiology 25:1454-1466 (1994); Stahl and Yancopoulos, Cell 74:587-590 (1993).

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FIGURE 7: Design of heterodimeric receptor-based ligand traps for IL-6. The heterodimeric ligand trap is comprised of two interdisulfide linked proteins, gp130-Fc and IL-6Rα-Fc. The gp130-Fc•IL-6Rα-Fc complex (upper panel) is shown to mimic the high affinity cytokine•Rα•β1 complex (lower panel). The ligand trap functions as an antagonist by sequestering IL-6 and thus rendering unavailable to interact with the native receptors on IL-6-responsive cells.

FIGURE 8. Heteromeric immunoglobulin Heavy/Light Chain Receptor Fusions. An example of a heavy/light chain receptor fusion molecule is schematically depicted. The extracellular domain of gp130 is fused to Cγ, whereas the extracellular domain of IL-6Rα is fused to the constant region of the kappa chain (κ). The inter-chain disulfide bridges are also depicted (S-S).

FIGURE 9. Amino acid sequence of gp130-Cγ1. Key: Amino acids 1 to 619 are from human gp130 (Hibi, et al., Cell 63:1149-1157 (1990). Ser-Gly bridge is shown in bold type. Amino acids 662 to 651 are from the constant region of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (*) shows the position of the STOP codon.

FIGURE 10: Amino acid sequence of gp130Δ3fibro. Key: Amino acids 1 to 330 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Other symbols as described in Figure 9.

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FIGURE 11: Amino acid sequence of J-CH1. Key: The Ser-Gly bridge is shown in bold, the J-peptide is shown in italics, the CH1 domain is underlined.

FIGURE 12: Amino acid sequence of Cγ4. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 239 comprise the Cγ4 sequence.

FIGURE 13: Amino acid sequence of κ-domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 108 comprise the κ domain. The C-terminal cysteine (amino acid 108) is that involved in the disulfide bond of the κ domain with the CH1 domain of Cγ.

FIGURE 14: Amino acid sequence of λ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 106 comprise the λ domain (Cheung, et al., J. Virol. 66: 6714-6720 (1992). The C-terminal cysteine (amino acid 106) is that involved in the disulfide bond of the λ domain with the CH1 domain of C γ .

15 FIGURE 15: Amino acid sequence of the soluble IL-6Rα domain. Key:
Amino acids 1 to 358 comprise the soluble IL-6Rα domain (Yamasaki, et al., Science 241:825-828 (1988). The Ala-Gly bridge is shown in bold type.

FIGURE 16: Amino acid sequence of the soluble IL-6Rα313 domain: Key:

20 Amino acids 1 to 313 comprise the truncated IL-6Rα domain (IL-6Rα313).

The Thr-Gly bridge is shown in bold type.

FIGURE 17: Purification of gp130-Cγ1•IL-6Rα-κ. 4% to 12% SDS-PAGE gradient gel run under non-reducing conditions. Proteins were visualized by staining with silver. Lane 1: approximately 100 ng of material purified over Protein A Sepharose (Pharmacia). Lane 2: Molecular size standards (Amersham). Lane 3: The Protein A-purified material shown here after further purification over an IL-6 affinity chromatography step. The positions of the gp130-Cγ1 dimer [(gp130-Cγ1)2], the gp130-Cγ1 dimer

associated with one IL-6R α - κ [(gp130-C γ 1)2•(IL-6R α - κ)1], and the gp130-C γ 1 dimer associated with two IL-6R α - κ [(gp130-C γ 1)2•(IL-6R α - κ)2] are shown, as well as the sizes for the molecular size standards in kilodaltons (200, 100, and 46).

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FIGURE 18: IL-6 dissociates slowly from the ligand trap. The dissociation rate of IL-6 from a heavy/light chain receptor-based ligand trap (gp130-C γ 1 \bullet IL-6R α - κ) was compared to that obtained with the neutralizing monoclonal antibody B-E8 (BE8 MAb).

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FIGURE 19: IL-6 can induce multimerization of the ligand trap. (A) Two different ligand traps are depicted schematically and listed according to their ability to bind protein A. gp130-Fc \bullet IL-6R α -Fc (GF6F) binds protein A via its Fc-domains, whereas gp130-CH1 \bullet IL-6R α - κ (G16K) does not bind to protein A. (B) Anti-kappa western blotting of proteins precipitated with Protein A-Sepharose from mixtures of GF6F \pm IL-6, G16K \pm IL-6, or GF6F plus G16K \pm IL-6, as marked.

FIGURE 20: Inhibition of IL-6-dependent XG-1 cell proliferation. XG-1 cells [Zhang, et al., Blood 83:3654-3663 (1994)] were prepared for a proliferation assay by starving the cells from IL-6 for 5 hours. Assays were set up in 96-well tissue culture dishes in RPMI + 10% fetal calf serum + penicillin/streptomycin + 0.050 nM 2-mercaptoethanol + glutamine. 0.1 ml of that media was used per well. Cells were suspended at a density of 250,000 per ml at the start of the assay. 72 hours post addition of IL-6 ± ligands traps or antibodies, an MTT assay was performed as described (Panayotatos et al. Biochemistry 33:5813-5818 (1994). The different ligand traps utilized are listed.

FIGURES 21A-21D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 424 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

- 5 FIGURES 22A-22D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 603 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.
- FIGURES 23A-23D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 622 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.
- FIGURE 24A-24F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 412 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.
 - FIGURE 25A-25F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 616 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

- FIGURE 26A-26E: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 569 which is capable of binding the cytokine IL-1 to form a nonfunctional complex.
- 25 FIGURE 27: Shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.
- FIGURE 28: Shows that an IL-4 trap designated 4SC375 displays

 30 antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap

 designated 4SC424 (described in Figs. 21A-21D) which is a fusion

polypeptide of IL-2R γ -IL4R α -Fc Δ C1 having the IL-2R γ component flush with the IL-4R α component.

- FIGURE 29: Shows that the IL6 trap (6SC412 IL6R-scb-gpx-FcΔC1) described in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 BE8.
- FIGURE 30: Shows that the trap 1SC569 (described in Figs. 26A-26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1.
 - FIGURE 31A-31G: The nucleotide and encoded amino acid sequence of the IL-4R α .IL-13R α 1.Fc single chain trap construct is set forth.
- 15 FIGURE 32A-32G: The nucleotide and encoded amino acid sequence of the IL-13Rα1.IL-4Rα.Fc single chain trap construct is set forth.
- FIGURE 33: Blocking of IL-13 by IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM. At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%.
- FIGURE 34: Blocking of IL-4 by IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc.

 Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc at a

 concentration of 10nM blocks IL-4-induced growth up to ~1nM. At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%.
 - FIGURE 35: Human IL-1 trap blocks the in vivo effects of exogenously administered huIL-1. BALB/c mice were given subcutaneous injection of huIL-1 (0.3 μ g/kg) at time 0. Twenty-four hours prior to huIL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess

of huIL-1 trap. Two hours prior to sacrifice (26 hrs), the mice were rechallenged with a second injection of huIL-1 (0.3 µg/kg, s.c.). Blood samples were collected at various time points and sera were assayed for IL-1 levels (expressed as mean +/- SEM; n=5 per group).

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FIGURE 36A & FIGURE 36B: Human IL-4 trap antagonizes the effects of human IL-4 in monkeys. Figure 36A: Cynomologus monkeys were treated in three parts as indicated. Human IL-4 (25 μ g/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Plasma was collected daily and assayed for MCP-1 levels. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.0007; Tukey-Kramer: Part 2 vs. Part 1, p,0.05; Part 2 vs. Part 3, p,0.05; Part 1 vs. Part 3, not significant.) Figure 36B: Cynomologus monkeys were treated in three parts as indicated. Human IL-4 (25 $\mu g/kg$) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.042; Tukey-Kramer: Part 2 vs. Part 1, p<0.05; Part 2 vs. Part 3 and Part 1 vs. Part 3, not significant.)

FIGURE 37: Murine IL-4 trap partially prevented IL-4-mediated IgE increase in mice. BALB/C mice injected with anti-mouse IgD
25 (100μl/mouse, s.c.) were randomly divided into 3 groups, each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Sera were collected at various time points and assayed for IgE levels. Results were expressed as mean+/-SEM (n=5 per group). (ANOVA p=0.0002; Tukey-Kramer: vehicle vs. IL-4 trap, p<0.01; vehicle vs. IL-4 antibody, p<0.001; IL-4 trap vs. IL-4 antibody, not significant).

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DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:

- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
- b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
- c) a nucleotide sequence encoding a third fusion polypeptide
 15 component comprising the amino acid sequence of a multimerizing component.

By "cytokine binding portion" what is meant is the minimal portion of the extracellular domain necessary to bind the cytokine. It is accepted by those of skill in the art that a defining characteristic of a cytokine receptor is the presence of the two fibronectin-like domains that contain canonical cysteines and of the WSXWS box (Bazan, J.F., 1990, PNAS 87: 6934-6938). Sequences encoding the extracellular domains of the binding component of the cytokine's receptor and of the signal transducing component of the cytokine's receptor may also be used to create the fusion polypeptide of the invention. Similarly, longer sequences encoding larger portions of the components of the cytokine's receptor may be used. However, it is contemplated that fragments smaller than the extracellular domain will function to bind the cytokine and therefore, the invention contemplates fusion polypeptides comprising the minimal portion of the extracellular domain necessary to bind the cytokine as the cytokine binding portion.

The invention comprises a "specificity determining component" of a cytokine's receptor and a "signal transducing component" of the cytokine's receptor. Regardless of the nomenclature used to designate a particular component or subunit of a cytokine receptor, one skilled in the art would recognize which component or subunit of a receptor is responsible for determining the cellular target of the cytokine, and thus would know which component constitutes the "specificity determining component."

Similarly, regardless of the nomenclature used, one of skill in the art would know which component or subunit of a receptor would constitute the "signal transducing component." As used herein, the "signal transducing component" is a component of the native receptor which is not the specificity determining component and which does not bind or weakly binds the cytokine in the absence of the specificity determining component. In the native receptor, the "signal transducing component" may participate in signaling.

For example, while some cytokine receptors have components designated α and β , the IL-4 receptor has a signal transducing component referred to as IL-2R γ . However, regardless of what name is associated with that component, one skilled in the art would know which component of the IL-4 receptor is the signal transducing component. Thus to practice the present invention and create a high affinity trap for IL-4, one of skill in the art would create an isolated nucleic acid comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the IL-4 receptor (IL-4R α); a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the IL-4 receptor (IL-2R γ); and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a

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multimerizing component (for example, an Fc domain of IgG) to create a high affinity trap for IL-4.

Some further examples of the receptor components that may be used to

5 prepare cytokine antagonists according to the invention are set forth in
Table 1. The Table 1 sets forth, by way of example but not by way of
limitation, some of the varied nomenclature used in the scientific
literature to describe those components which function as specificity
determining components and those which function as signal transducing

10 components of certain cytokine receptors.

TABLE 1

Cytokine	Specificity determining Component	Signal transducing Component
Interleukin-1 (IL-1)	Type I IL-IR (ref. 8) Type II IL-IR (ref. 8) IL-IRI (ref. 11) IL-IRII (ref. 11)	IL-1R AcP (refs. 8, 11)
Interleukin-2 (IL-2)	α -subunit (ref. 2) α -chain (ref. 3) IL-2R α (ref. 1)	β-chain (ref. 3) β-subunit (ref. 2) γ-chain (ref. 3) IL-2Rβ (refs. 1, 10) IL-2Rγ (refs. 1, 10)
Interleukin-3 (IL-3)	IL-3R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)
Interleukin-4 (IL-4)	IL-4R (ref. 1)	γ -chain (ref. 3) IL-2R γ (ref. 1)
Interleukin-5 (IL-5)	IL-5R α (ref. 1) α -subunit (ref. 2) α -receptor component (ref. 5)	β _c (ref. 1) β-subunit (ref. 2) β-chain (ref. 3) β-receptor component (ref. 5)

TABLE 1 (CONT'D)

Cytokine	Specificity determining Component	Signal transducing Component
Granulocyte macrophage-colony stimulating factor (GM-CSF)	α-receptor component (ref. 5) α-subunit (ref. 2) GMRα (refs. 1, 2)	β-receptor component (ref. 5) β-subunit (ref. 2) β-chain (ref. 3) β _C (ref. 1) GMRβ (refs. 1, 2)
Leukemia inhibitory factor (LIF)	LIFBP (ref. 1) α -receptor component (ref. 5)	gp130 (refs. 1, 3) β- receptor component (ref. 5)
Interleukin-11 (IL-11)	α–chain (ref. 4) NR1 (ref. 4)	gp130 (ref. 4)
Interleukin-15 (IL-15)	IL-15R α (ref. 10)	IL-2Rβ (ref. 10) IL-2Rγ (ref. 10)
Interferon-y (IFNy)	IFN-γR (ref. 7) IFN-γR1 (ref. 7)	AF-1 (ref. 7) IFN-γR2 (ref. 7)
ТСFβ	Type II (refs. 6, 9)	Type I (refs. 6, 9)

Only a few of the multitude of references are cited in Table 1, and they are set forth as follows:

- 1. Sato and Miyajima, Current Opinions in Cell Biology 6: 174-179
- 5 (1994) See page 176, lines 9-16;
 - 2. Miyajima, et al., Annual Review of Immunology 10: 295-331 (1992) See page 295, line 4 to page 296, line 1; page 305, last paragraph;
 - 3. Kondo, et al, Science 262: 1874-1877 (1993) See page 1874, cols. 1 & 2;
 - 4. Hilton, et al, EMBO Journal 13: 4765-4775 (1994) See page 4766, col.
- 10 1, lines 20 24;
 - 5. Stahl and Yancopoulos, Cell 74: 587-590 (1993) See page 587, column 2, lines 15-22;
 - 6. Bassing, et al, Journal of Biological Chemistry 269: 14861-14864 (1994)
 See page 14861, col. 2, lines 1-9 and 21-28;
- 7. Kotenko, et al, Journal of Biological Science 270: 20915-20921 (1995) See page 20915, lines 1-5 of the abstract;
 - 8. Greenfeder, et al., Journal of Biological Chemistry 270: 13757-13765 (1995) See page 13757, col. 1, line 6 to col. 2, line 3 and col. 2, lines 10-12; page 13764, col. 2, last 3 lines and page 13765, col. 1, lines 1-7;
- 20 9. Lebrun and Vale, Molecular Cell Biology 17: 1682-1691 (1997) See page 1682, Abstract lines 2-6;
 - 10. Kennedy and Park, Journal of Clinical Immunology 16: 134-143 (1996) See page 134, lines 1-7 of the abstract; page 136, col 2., lines 1-5;
 - 11. Wesche, et al., Journal of Biological Chemistry 272: 7727-7731 (1997)
- 25 See page 7731, lines 20-26.

Kotenko, et al. recently identified the IL-10R2 (IL-10Rβ) chain which is reported to serve as an accessory chain that is essential for the active IL-10 receptor complex and for initiating IL-10 induced signal transduction

events (S.V. Kotenko, et al., The EMBO Journal, 1997, Vol. 16: 5894-5903).

Additional cytokines and their receptors are described in Appendix II, page
A:9 of Immunobiology, The Immune System In Health and Disease, 2nd

Edition, by Charles A. Janeway, Jr. and Paul Travers, published by Current Biology Ltd./Garland Publishing Inc., copyright 1996.

In preparing the nucleic acid sequence encoding the fusion polypeptide of the invention, the first, second, and third components of the fusion 5 polypeptide are encoded in a single strand of nucleotides which, when expressed by a host vector system, produces a monomeric species of the fusion polypeptide. The monomers thus expressed then multimerize due to the interactions between the multimerizing components (the third fusion polypeptide components). Producing the fusion polypeptides in 10 this manner avoids the need for purification of heterodimeric mixtures that would result if the first and second components were produced as separate molecules and then multimerized. For example, U.S. Patent No. 5.470,952 issued November 28, 1995 describes the production of heterodimeric proteins that function as CNTF or IL-6 antagonists. The 15 heterodimers are purified from cell lines cotransfected with the appropriate alpha (α) and beta (β) components. Heterodimers are then separated from homodimers using methods such as passive elution from preparative, nondenaturing polyacrylamide gels or by using high pressure cation exchange chromatography. The need for this purification step is 20 avoided by the methods of the present invention.

In addition, PCT International Application WO 96/11213 published 18 April 1996 entitled Dimeric IL-4 Inhibitors states that the applicant has prepared homodimers in which two IL-4 receptors are bound by a polymeric spacer and has prepared heterodimers in which an IL-4 receptor is linked by a polymeric spacer to an IL-2 receptor gamma chain. The polymeric spacer described is polyethylene glycol (PEG). The two receptor components, IL-4R and IL-2Rgamma are separately expressed and purified. Pegylated homodimers and heterodimers are then produced by joining the components together using bi-functional PEG reagents. It is an advantage

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of the present invention that it avoids the need for such time consuming and costly purification and pegylation steps.

In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the invention may be prepared in which the order of the first, second and third fusion polypeptide components are rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, leukemia inhibitory factor, and cardiotrophin-1.

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In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

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In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the immunoglobulin superfamily

of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

In still further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18, and MIF.

Because specificity determination and signal transduction occurs by a

similar mechanism in the TGF-β/BMP family of cytokines (See D.

Kingsley, Genes & Development, 1994, 8: 133-146; J. Wrana, Miner

Electrolyte Metab, 24: 120-130 (1998); R. Derynck and X. Feng, Biochimica et

Biophysica Acta 1333 (1997) F105-F150; and J. Massague and F. Weis-Garcia,

"Serine/threonine Kinase Receptors: Mediators of Transforming Growth

Factor Beta Family Signals" In Cancer Surveys, Vol. 27: Cell Signaling,

1996, Imperial Cancer Research Fund) the present invention may be used
to produce high affinity antagonists for cytokines that are members of the

TGF-β/BMP family.

Therefore, in additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TGF-β/BMP family selected from the group consisting of TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian

inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

In alternative embodiments of the invention, the specificity determining component, the signal transducing component, or both, may be substituted 5 for by a single chain Fv. A single chain Fv (scFv) is a truncated Fab having only the V region of a heavy chain linked by a stretch of synthetic peptide to a V region of a light chain. See, for example, US Patent Nos. 5,565,332; 5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge Antibody Technology Limited incorporated by reference herein. Thus the 10 present invention contemplates, for example, an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the 15 specificity determining component of the cytokine's receptor; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of an scFv capable of binding the cytokine at a site different from the site at which the cytokine binding portion of the extracellular domain of the specificity determining component of the 20 cytokine's receptor binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component. Alternatively, the specificity determining component may be substituted for by a scFv that binds to a site on the cytokine different from the site at which the signal transducing 25 component binds. Thus the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a scFv that binds to a site on the cytokine different from the 30 site at which the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor binds; a nucleotide sequence encoding a second fusion polypeptide component

comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In another embodiment, the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a first scFv that binds to a site on the cytokine; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence a second scFv that binds to a site on the cytokine different from the site at which the first scFv binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In all of the above described embodiments comprising scFv's, the invention also contemplates embodiments in which the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component; embodiments in which the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component; and further embodiments of the invention in which the order of the first, second and third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

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In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(Δ C1). Alternatively, the multimerizing component may be an Fc domain in which a cysteine within the first five amino acids has been substituted for by another amino acid such as, for example, serine or alanine.

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The present invention also provides for fusion polypeptides encoded by the isolated nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the third multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the third multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion

polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera</u> frugiperda, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

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The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

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The present invention provides novel antagonists which are based on receptor components that are shared by cytokines such as the CNTF family of cytokines.

The invention described herein contemplates the production of antagonists to any cytokine that utilizes an α specificity determining component which, when combined with the cytokine, binds to a first β signal transducing component to form a nonfunctional intermediate which then binds to a second β signal transducing component causing β-receptor dimerization and consequent signal transduction. According to the invention, the soluble α specificity determining component of the receptor (sRα) and the extracellular domain of the first β signal transducing component of the cytokine receptor (β1) are combined to form heterodimers (sRα:β1) that act as antagonists to the cytokine by binding the

As described in Example 1, CNTF and IL-6 share the $\beta 1$ receptor component gp130. The fact that CNTF forms an intermediate with CNTFR α and gp130 can be demonstrated (Example 1) in cells lacking LIFR β , where the complex of CNTF and CNTFR α binds gp130, and

cytokine to form a nonfunctional complex.

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prevents homodimerization of gp130 by IL-6 and IL-6Rα, thereby blocking signal transduction. These studies provide the basis for the development of the IL-6 antagonists described herein, as they show that if, in the presence of a ligand, a nonfunctional intermediate complex, consisting of the ligand, its α receptor component and its β1 receptor component, can be formed, it will effectively block the action of the ligand. Other cytokines may use other β1 receptor components, such as LIFRβ, which may also be used to produce antagonists according to the present invention.

- Thus for example, in one embodiment of the invention, effective antagonists of IL-6 or CNTF consist of heterodimers of the extracellular domains of the α specificity determining components of their receptors (sIL-6Rα and sCNTFRα, respectively) and the extracellular domain of gp130. The resultant heterodimers, which are referred to hereinafter as sIL-6Rα:β1 and sCNTFRα:β1, respectively, function as high-affinity traps for IL-6 or CNTF, respectively, thus rendering the cytokine inaccessible to form a signal transducing complex with the native membrane-bound forms of their receptors.
- Although soluble ligand binding domains from the extracellular portion of receptors have proven to be somewhat effective as traps for their ligands and thus act as antagonists [Bargetzi, et al., Cancer Res. 53:4010-4013 (1993); , et al., Proc. Natl. Acad. Sci. USA 89: 8616-8620 (1992); Mohler, et al., J. Immunol. 151: 1548-1561 (1993); Narazaki, et al., Blood 82: 1120-1126 (1993)], the IL-6 and CNTF receptors are unusual in that the α receptor
 - components constitute ligand binding domains that, in concert with their ligands, function effectively in soluble form as receptor agonists [Davis, et al. Science 259:1736-1739 (1993); Taga, et al., Cell 58: 573-581 (1989)]. The sRα:β1 heterodimers prepared according to the present invention provide effective traps for their ligands, binding these ligands with affinities in the picomolar range (based on binding studies for CNTF to PC12D cells)

without creating functional intermediates. The technology described herein may be applied to develop a cytokine trap for any cytokine that utilizes an α -component that confers specificity, as well as a β component which, when bound to the α -specificity component, has a higher affinity 5 for the cytokine than either component alone. Accordingly, antagonists according to the invention include antagonists of interleukins 1 through 5 [IL-1, Greenfeder, et al. J. Biol. Chem. 270:13757-13765 (1995); Guo, et al. J. Biol. Chem. 270:27562-27568 (1995)], IL-2; [Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 10 (1992)]; IL-3; [Kitamura, et al. Cell 66:1165-1174 (1991)], IL-4; [Idzerda, et al. J. Exp. Med. 171:861-873 (1990)], IL-5; [Taverneir, et al. Cell 66:1175-1184 (1991)], IL-11 [(Cherel, et al. Direct Submission to EMBL/GenBank/DDBI databases; accession No. Z38102)], interleukin 15 [IL-15; Hemar, et al. J. Cell Biol. 1295:55-64 (1995); Taniguchi, et al. European Patent Nos. 0386289-A 15 and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)], granulocyte-macrophage colony stimulating factor [GM-CSF; Hayashida, et al. Proc. Natl. Acad. Sci. U.S.A. 97:9655-9659 (1990)], LIF, gamma interferon [IFNy; Aguet, et al. Cell 55:273-280 (1988); Soh, et al. Cell 76:793-802 (1994)], and transforming growth factor beta [TGFβ; Inagaki, et al. Proc. Natl. Acad. Sci. USA 90:5359-5363 (1993)]. 20

The α and β receptor extracellular domains may be prepared using methods known to those skilled in the art. The CNTFR α receptor has been cloned, sequenced and expressed [Davis, et al. (1991) Science 253:59-63 which is incorporated by reference in its entirety herein]. The cloning of LIFR β and gp130 are described in Gearing et al. in EMBO J. 10:2839-2848 (1991), Hibi, et al. Cell 63:1149-1157 (1990) and in published PCT application WO 93/10151 published May 27, 1993, all of which are incorporated by reference in their entirety herein.

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The receptor molecules useful for practicing the present invention may be prepared by cloning and expression in a prokaryotic or eukaryotic expression system. The recombinant receptor gene may be expressed and purified utilizing any number of methods. The gene encoding the factor may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

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The recombinant factors may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The sRα:β heterodimeric receptors may be engineered using known fusion regions, as described in published PCT application WO 93/10151 published May 27, 1993 entitled "Receptor for Oncostatin M and Leukemia Inhibitory Factor" which describes production of β receptor heterodimers, or they may be prepared by crosslinking of extracellular domains by chemical means. The domains utilized may consist of the entire extracellular domain of the α and β components, or they may consist of mutants or fragments thereof that maintain the ability to form a complex with its ligand and other components in the sRα:β1 complex. For example, as described below in Example 4, IL-6 antagonists have been prepared using gp130 that is lacking its three fibronectin-like domains.

In one embodiment of the invention, the extracellular domains are engineered using leucine zippers. The leucine zipper domains of the human transcription factors c-jun and c-fos have been shown to form stable heterodimers [Busch and Sassone-Corsi, Trends Genetics 6: 36-40]

(1990); Gentz, et al., Science 243: 1695-1699 (1989)] with a 1:1 stoichiometry. Although jun-jun homodimers have also been shown to form, they are about 1000-fold less stable than jun-fos heterodimers. Fos-fos homodimers have not been detected.

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The leucine zipper domain of either c-jun or c-fos are fused in frame at the C-terminus of the soluble or extracellular domains of the above mentioned receptor components by genetically engineering chimeric genes. The fusions may be direct or they may employ a flexible linker domain, such as the hinge region of human IgG, or polypeptide linkers consisting of small amino acids such as glycine, serine, threonine or alanine, at various lengths and combinations. Additionally, the chimeric proteins may be tagged by His-His-His-His-His-His (His6),[SEQ. ID NO. 1] to allow rapid purification by metal-chelate chromatography, and/or by epitopes to which antibodies are available, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

In another embodiment, as described below in Example 3, the $sR\alpha$: β 1 heterodimer is prepared using a similar method, but using the Fc-domain 20 of human IgG1 [Aruffo, et al., Cell 67:35-44 (1991)]. In contrast to the latter, formation of heterodimers must be biochemically achieved, as chimeric molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers. Thus, homodimers may be reduced under conditions that 25 favor the disruption of inter-chain disulfides but do not effect intra-chain disulfides. Then monomers with different extracellular portions are mixed in equimolar amounts and oxidized to form a mixture of homoand heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of 30 heterodimers may be biased by genetically engineering and expressing molecules that consist of the soluble or extracellular portion of the receptor components followed by the Fc-domain of hIgG, followed by

either the c-jun or the c-fos leucine zippers described above [Kostelny, et al., J. Immunol. 148: 1547-1553 (1992)]. Since these leucine zippers form predominately heterodimers, they may be used to drive formation of the heterodimers where desired. As for the chimeric proteins described using leucine zippers, these may also be tagged with metal chelates or an epitope. This tagged domain can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

- 10 In additional embodiments, heterodimers may be prepared using other immunoglobulin derived domains that drive the formation of dimers. Such domains include, for example, the heavy chains of IgG (Cy1 and Cy4), as well as the constant regions of kappa (κ) and lambda (λ) light chains of human immunoglobulins. The heterodimerization of Cy with the light 15 chain occurs between the CH1 domain of Cy and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. Accordingly, as described in Example 4, constructs may be prepared using these immunoglobulin domains. Alternatively, the immunoglobulin domains include domains that may 20 be derived from T cell receptor components which drive dimerization. In another embodiment of the invention, the $sR\alpha:\beta 1$ heterodimers are prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two soluble or extracellular domains fused together in 25 tandem ("head to head") by a flexible loop. This loop may be entirely artificial (e.g. polyglycine repeats interrupted by serine or threonine at a certain interval) or "borrowed" from naturally occurring proteins (e.g. the hinge region of hIgG). Molecules may be engineered in which the order of the soluble or extracellular domains fused is switched (e.g.
- $sIL6R\alpha/loop/sgp130$ or $sgp130/loop/sIL-6R\alpha)$ and/or in which the length

and composition of the loop is varied, to allow for selection of molecules with desired characteristics.

Alternatively, the heterodimers made according to the present invention may be purified from cell lines cotransfected with the appropriate α and β components. Heterodimers may be separated from homodimers using methods available to those skilled in the art. For example, limited quantities of heterodimers may be recovered by passive elution from preparative, nondenaturing polyacrylamide gels. Alternatively, heterodimers may be purified using high pressure cation exchange chromatography. Excellent purification has been obtained using a Mono S cation exchange column.

In addition to sRα:β1 heterodimers that act as antagonists by binding free CNTF or IL-6, the present invention also contemplates the use of engineered, mutated versions of IL-6 with novel properties that allow it to bind to IL-6R α and a single gp130 molecule, but fail to engage the second gp130 to complete β component homodimerization, and thus act as an effective IL-6 antagonist on any IL-6 responsive cell. Our model for the structure of the IL-6 and CNTF receptor complexes indicates that these cytokines have distinct sites for binding the α, β1, and β2 receptor components [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Mutations of critical amino acid residues comprising each of these sites gives rise to novel molecules which have the desired antagonistic properties. Ablation of the $\beta 1$ site would give a molecule which could still bind to the α receptor component but not the $\beta1$ component, and thereby comprise an antagonist with nanomolar affinity. Mutations of critical amino acid residues comprising the β2 site of IL-6 (IL-6β2-) would give a molecule that would bind to IL-6R α and the first gp130 monomer, but fail to engage the second gp130 and thus be functionally inactive. Similarly, mutations of

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the CNTF $\beta 2$ site would give a molecule (CNTF $\beta 2$ -) that would bind CNTFR α and gp130, but fail to engage LIFR β , thereby antagonizing CNTF action by forming the non-functional $\beta 1$ intermediate. Based on the binding results described above where CNTF forms the $\beta 1$ intermediate with high affinity, both CNTF $\beta 2$ - and IL-6 $\beta 2$ - would constitute antagonists with affinity in the range of 10 pM.

A variety of means are used to generate and identify mutations of IL-6 or CNTF that have the desired properties. Random mutagenesis by standard methods of the DNA encoding IL-6 or CNTF may be used, followed by analysis of the collection of products to identify mutated cytokines having the desired novel properties as outlined below. Mutagenesis by genetic engineering has been used extensively in order to elucidate the structural organization of functional domains of recombinant proteins. Several different approaches have been described in the literature for carrying out deletion or substitution mutagenesis. The most successful appear to be alanine scanning mutagenesis [Cunningham and Wells (1989), Science 244: 1081-1085] and homolog-scanning mutagenesis [Cunningham, et al., (1989), Science 243:1330-1336].

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Targeted mutagenesis of the IL-6 or CNTF nucleic acid sequences using such methods can be used to generate CNTF β 2- or IL-6 β 2- candidates. The choice of regions appropriate for targeted mutagenesis is done systematically, or determined from studies whereby panels of monoclonal antibodies against each factor are used to map regions of the cytokine that might be exposed after binding of the cytokine to the α receptor component alone, or to the $\alpha\beta$ 1 heterodimeric soluble receptors described above. Similarly, chemical modification or limited proteolysis of the cytokine alone or in a complex bound to the α receptor component or the $\alpha\beta$ 1 heterodimeric soluble receptors described above, followed by analysis

of the protected and exposed regions could reveal potential $\beta 2$ binding sites.

Assays for identifying CNTF or IL-6 mutants with the desired properties involve the ability to block with high affinity the action of IL-6 or CNTF on appropriately responsive cell lines [Davis, et al., Science 259: 1736-1739 (1993); Murakami, et al., Proc. Natl. Acad. Sci. USA 88: 11349-11353 (1991)]. Such assays include cell proliferation, survival, or DNA synthesis driven by CNTF or IL-6, or the construction of cell lines where binding of factor induces production of reporters such as CAT or β -galactosidase [Savino, et al., Proc. Natl. Acad. Sci. USA 90: 4067-4071 (1993)].

Alternatively, the properties of various mutants may be assessed with a receptor-based assay. One such assay consists of screening mutants for their ability to bind the sR α : β 1 receptor heterodimers described above using epitope-tagged [Davis et al., Science 253: 59-63 (1991)] sR α : β 1 reagents. Furthermore, one can probe for the presence or absence of the β 2 site by assessing whether an epitope-tagged soluble β 2 reagent will bind to the cytokine in the presence of the β 1 heterodimer. For example, CNTF only binds to LIFR β (the β 2 component) in the presence of both CNTFR α and gp130 [Davis, et al. Science 260: 1805-1808 (1993); Stahl, et al. J. Biol. Chem. 268: 7628-7631 (1993)]. Thus a soluble LIFR β reagent would only bind to CNTF in the presence of the soluble sR α : β 1 dimer sCNTFR α : β 1. For IL-6, the sR α : β 1 reagent would be IL-6R α : β 1, and the probe for the β 2 site would be epitope-tagged sgp130. Thus β 2- mutants of CNTF would be identified as those that bound the sR α : β 1 reagent, demonstrating that the α and β 1 site of the cytokine were intact, yet failed to bind the β 2 reagent.

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In addition, the present invention provides for methods of detecting or measuring the activity of potential β 2- mutants by measuring the phosphorylation of a β -receptor component or a signal transduction component selected from the group consisting of Jak1, Jak2 and Tyk2 or any other signal transduction component, such as the CLIPs, that are determined to be phosphorylated in response to a member of the CNTF family of cytokines.

A cell that expresses the signal transduction component(s) described

herein may either do so naturally or be genetically engineered to do so.

For example, Jak1 and Tyk-2-encoding nucleic acid sequences obtained as described in Velazquez, et al., Cell, Vol. 70:313-322 (1992), may be introduced into a cell by transduction, transfection, microinjection, electroporation, via a transgenic animal, etc., using any known method known in the art.

According to the invention, cells are exposed to a potential antagonist and the tyrosine phosphorylation of either the β -component(s) or the signal transduction component(s) are compared to the tyrosine phosphorylation of the same component(s) in the absence of the potential antagonist. In another embodiment of the invention, the tyrosine phosphorylation that results from contacting the above cells with the potential antagonist is compared to the tyrosine phosphorylation of the same cells exposed to the parental CNTF family member. In such assays, the cell must either express the extracellular receptor (α -component) or the cells may be exposed to the test agent in the presence of the soluble receptor component. Thus, for example, in an assay system designed to identify agonists or antagonists of CNTF, the cell may express the α - component CNTFR α , the β -components gp130 and LIFR β and a signal transducing component such as Jak1. The cell is exposed to test agents, and the tyrosine phosphorylation of either the β - components or the signal transducing component is

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compared to the phosphorylation pattern produced in the presence of CNTF. Alternatively, the tyrosine phosphorylation which results from exposure to a test agent is compared to the phosphorylation which occurs in the absence of the test agent. Alternatively, an assay system, for example, for IL-6 may involve exposing a cell that expresses the β -component gp130 and a signal transducing protein such as Jak1, Jak2 or Tyk2 to a test agent in conjunction with the soluble IL-6 receptor.

In another embodiment of the invention the above approaches are used to 10 develop a method for screening for small molecule antagonists that act at various steps in the process of ligand binding, receptor complex formation, and subsequent signal transduction. Molecules that potentially interfere with ligand-receptor interactions are screened by assessing interference of complex formation between the soluble receptors and ligand as described 15 above. Alternatively, cell-based assays in which IL-6 or CNTF induce response of a reporter gene are screened against libraries of small molecules or natural products to identify potential antagonists. Those molecules showing antagonist activity are rescreened on cell-based assays responding to other factors (such as GM-CSF or factors like Neurotrophin-3 that activate receptor tyrosine kinases) to evaluate their specificity against 20 the CNTF/IL-6/OSM/LIF family of factors. Such cell-based screens are used to identify antagonists that inhibit any of numerous targets in the signal transduction process.

In one such assay system, the specific target for antagonists is the interaction of the Jak/Tyk family of kinases [Firmbach-Kraft, Oncogene 5: 1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11:2057-2065 (1991)] with the receptor β subunits. As described above, LIFRβ and gp130 preassociate with members of the Jak/Tyk family of cytoplasmic protein tyrosine kinases, which become activated in response to ligand-induced β component dimerization Stahl, et al. Science 263:92-95 (1993). Thus small molecules that could enter the cell cytoplasm and disrupt the interaction

between the β component and the Jak/Tyk kinase could potentially block all subsequent intracellular signaling. Such activity could be screened with an in vitro scheme that assessed the ability of small molecules to block the interaction between the relevant binding domains of purified β component and Jak/Tyk kinase. Alternatively, one could easily screen for molecules that could inhibit a yeast-based assay of β component binding to Jak/Tyk kinases using the two-hybrid interaction system [Chien, et al., Proc. Natl. Acad. Sci. 88: 9578-9582 (1991)]. In such a system, the interaction between two proteins (β component and Jak/Tyk kinase or relevant domains thereof in this example) induces production of a convenient marker such as β - galactosidase. Collections of small molecules are tested for their ability to disrupt the desired interaction without inhibiting the interaction between two control proteins. The advantage of this screen would be the requirement that the test compounds enter the cell before inhibiting the interaction between the β component and the Jak/Tyk kinase.

The CNTF family antagonists described herein either bind to, or compete with the cytokines CNTF and IL-6. Accordingly, they are useful for treating diseases or disorders mediated by CNTF or IL-6. For example, therapeutic uses of IL-6 antagonists would include the following:

1) In osteoporosis, which can be exacerbated by lowering of estrogen levels in post-menopausal women or through ovariectomy, IL-6 appears to be a critical mediator of osteoclastogenesis, leading to bone resorption [Horowitz, Science 260: 626-627 (1993); Jilka, et al., Science 257: 88-91 (1992)]. Importantly, IL-6 only appears to play a major role in the estrogen-depleted state, and apparently is minimally involved in normal bone maintenance. Consistent with this, experimental evidence indicates that function-blocking antibodies to IL-6 can reduce the number of osteoclasts [Jilka, et al. Science 257: 88-91 (1992)]. While estrogen replacement therapy is also used, there appear to be side effects that may include increased risk of

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endometrial and breast cancer. Thus, IL-6 antagonists as described herein would be more specific to reduce osteoclastogenesis to normal levels.

- 2) IL-6 appears to be directly involved in multiple myeloma by acting in either an autocrine or paracrine fashion to promote tumor formation [van Oers, et al., Ann Hematol. 66: 219-223 (1993)]. Furthermore, the elevated IL-6 levels create undesirable secondary effects such as bone resorption, hypercalcemia, and cachexia; in limited studies function-blocking antibodies to IL-6 or IL-6Ra have some efficacy [Klein, et al., Blood 78: 1198-1204 (1991); Suzuki, et al., Eur. J. Immunol. 22:1989-1993 (1992)]. Therefore, IL-6 antagonists as described herein would be beneficial for both the secondary effects as well as for inhibiting tumor growth.
- 3) IL-6 may be a mediator of tumor necrosis factor (TNF) that leads to cachexia associated with AIDS and cancer [Strassmann, et al., J. Clin. Invest. 89: 1681-1684 (1992)], perhaps by reducing lipoprotein lipase activity in adipose tissue [Greenberg, et al., Cancer Research 52: 4113-4116 (1992)]. Accordingly, antagonists described herein would be useful in alleviating or reducing cachexia in such patients.

Effective doses useful for treating these or other CNTF family related diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the antagonists described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation (including antagonist expressing cells) prior to administration *in vivo*. For example, the pharmaceutical composition may comprise one or more of the antagonists in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such

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treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

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Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

EXAMPLES

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EXAMPLE 1: CNTF COMPETES WITH IL-6 FOR BINDING TO GP130

MATERIALS AND METHODS

- Materials. A clone of PC12 cells that respond to IL-6 (PC12D) was obtained from DNAX. Rat CNTF was prepared as described [Masiakowski, et al., J. Neurochem. 57:1003-10012 (1991)]. IL-6 and sIL-6Rα were purchased from R & D Systems. Antisera was raised in rabbits against a peptide derived from a region near the C-terminus of gp130 (sequence:
- 25 CGTEGQVERFETVGME) [SEQ. ID. NO. 2] by the method described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993). Anti-phosphotyrosine monoclonal 4G10 was purchased from UBI, and reagents for ECL from Amersham.
- 30 <u>Signal Transduction Assays</u>. Plates (10 cm) of PC12D were starved in serum-free medium (RPMI 1640 + glutamine) for 1 hour, then incubated with IL-6 (50 ng/mL) + sIL-6R (1 mg/mL) in the presence or absence of

added rat CNTF at the indicated concentrations for 5 minutes at 37°C. Samples were then subjected to anti-gp130 immunoprecipitation, SDS PAGE, and anti-phosphotyrosine immunoblotting as described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993).

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RESULTS

The ability of CNTF to block IL-6 responses was measured using a PC12 cell line (called PC12D) that expresses IL-6Rα, gp130, and CNTFRα, but not LIFRB. As one would predict, these cells respond to IL-6, but not to CNTF (Fig. 2) since LIFRβ is a required component for CNTF signal transduction [Davis, et al., Science 260: 59-63 (1993)]. In accordance with results on other cell lines [Ip, et al., Cell 69: 1121-1132 (1992)], PC12D cells give tyrosine phosphorylation of gp130 (as well as a variety of other proteins called CLIPs) in response to 2 nM IL-6 (Fig. 2). Addition of recombinant soluble IL-6R α (sIL-6R α) enhances the level of gp130 tyrosine phosphorylation, as has been reported in some other systems [(Taga, et al., Cell 58: 573-581 (1989)]. However, addition of 2 nM CNTF simultaneously with IL-6 severely diminishes the tyrosine phosphorylation of gp130. Although a slight gp130 phosphorylation response remains in the presence of CNTF, IL-6, and sIL-6Rα, it is eliminated if the CNTF concentration is increased fourfold to 8 nM. Thus, in IL-6 responsive cells that contain CNTFRα but no LIFRβ, CNTF is a rather potent antagonist of IL-6 action.

25 EXAMPLE 2. BINDING OF CNTF TO THE CNTFRα:β

MATERIALS AND METHODS

Scatchard Analysis of CNTF Binding. 125I-CNTF was prepared and purified as described [Stahl et al. JBC 268: 7628-7631 (1993)]. Saturation binding studies were carried out in PC12 cells, using concentrations of 125I-

CNTF ranging from 20pM to 10nM. Binding was performed directly on a monolayer of cells. Medium was removed from wells and cells were washed once with assay buffer consisting of phosphate buffered saline (PBS; pH 7.4), 0.1mM bacitracin, 1mM PMSF, 1mg/ml leupeptin, and 1mg/ml BSA. Cells were incubated in 125I-CNTF for 2 hours at room temperature, followed by 2 quick washes with assay buffer. Cells were lysed with PBS containing 1% SDS and counted in a Packard Gamma Counter at 90-95% efficiency. Non-specific binding was defined by the presence of 100-fold excess of unlabelled CNTF. Specific binding ranged from 70% to 95%.

RESULTS

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The equilibrium constant for binding of CNTF to CNTFRα:β1 was 15 estimated from Scatchard analysis of iodinated CNTF binding on PC12D cells (Figure 3). The data is consistent with a 2 site fit having dissociation constants of 9 pM and 3.4 nM. The low affinity site corresponds to interaction of CNTF with CNTFR α , which has a Kd near 3 nM [(Panayotatos, et al., J. Biol. Chem. 268: 19000-19003 (1993)]. We interpret 20 the high affinity complex as the intermediate containing CNTF, CNTFR α , and gp130. A Ewing sarcoma cell line (EW-1) which does contain CNTFR α , gp130, and LIFR β , and therefore gives robust tyrosine phosphorylation in response to CNTF, displays a very similar two site fit with dissociation constants of 1 nM and 10. Thus it is apparent that CNTF binds with equally high affinity to a complex containing only CNTFRa 25 and gp130, as it does to a complex which additionally contains LIFRβ, thus demonstrating the feasibility of creating the sRα:β antagonists described herein.

EXAMPLE 3. METHODS OF PRODUCING CYTOKINE LIGAND TRAPS

Virus Stock Production

5 SF21 insect cells obtained from *Spodoptera frugiperda* were grown at 27°C in Gibco SF900 II medium to a density of 1x10⁶ cells/mL. The individual virus stock for either GP130-Fc-His6 (Figure 4) or IL6Ra-Fc (Figure 5) was added to the bioreactor to a low multiplicity 0.01-0.1 PFU/cell to begin the infection. The infection process was allowed to continue for 5-7 days allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically aliquoted into sterile centrifuge bottles and the cells removed by centrifugation. The cell-free supernatant was collected in sterile bottles and stored at 4°C until further use.

The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 2x10⁶ cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow the virus to adsorb to individual cells. An agar overlay is added and plates incubated for 5 - 7 days at 27°C. Staining of viable cells with neutral red revealed circular plaques resulting which were counted to give the virus titer.

Coinfection of Cells for Protein Production

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Uninfected SF21 Cells were grown in a 60L ABEC bioreactor containing 40L of SF900 II medium. Temperature was controlled at 27°C and the dissolved oxygen level was maintained at 50% of saturation by controlling the flowrate of oxygen in the inlet gas stream. When a density of 2x10⁶ cells/mL was reached, the cells were concentrated within the bioreactor to a volume of 20L using a low shear steam sterilizable pump with a tangential flow filtration device with Millipore Prostak 0.65 micron

membranes. After concentration fresh sterile growth medium is slowly added to the bioreactor while the filtration system continues to remove the spent growth medium by diafiltration. After two volume exchanges (40L) have been carried out an additional 20L of fresh medium was added to the bioreactor to resuspend the cells to the original volume of 40L. The cell density was determined once again by counting viable cells using a hemacytometer.

The required amount of each virus stock was calculated based on the cell

density, virus titer and the desired multiplicity of infection (MOI). Virus

stock ratios of 5:1, 5:2, 10:2 and 10:4, IL6Rα-Fc to GP130-Fc-His6 all resulted

in production of significant amounts of heterodimer. The ideal virus

stock ratio is highly dependent on the ease of purification of the

heterodimer from each of the two homodimers. The IL6Rα-Fc

15 homodimer is relatively easy to remove downstream by immobilized

metal affinity chromatography. Virus infection ratios have been chosen to

minimize the formation of the GP130-Fc-His6 homodimer which is more

difficult to clear downstream. The relative amount of GP130-Fc-His6 virus

stock chosen for infection has increased with successive batches as the

20 purification method for clearing the resultant homodimer has improved.

The virus stocks were aseptically mixed in a single vessel then transferred to the bioreactor. This results in synchronous infection of the SF21 cells. The infection is allowed to proceed for three to four days, allowing sufficient time for maximal production of the heterodimer protein.

Recovery and Protein A Chromatographic Purification

At the conclusion of the infection phase of the bioreactor process the cells were concentrated in the bioreactor using a 10 ft² Millipore Prostak filter (0.65 micron) pore size. The cell-free permeate passing through the filter was collected in a clean process vessel. At the conclusion of the filtration

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operation the pH of permeate stream, containing the protein product, was adjusted to 8.0 with 10N NaOH. The resultant precipitate was removed by forcing the extract through a 0.8 micron depth filter (Sartorious), followed by a 0.2 micron filter. Sufficient 0.5M EDTA stock was added to give a final concentration of 5mM. The filtered protein solution was loaded onto a 10 cm diameter column containing 100-200 mL of Pharmacia Protein A Sepharose 4 Fast Flow, equilibrated with PBS. Protein A has a very high affinity for the Fc-Fc domain of each of the 3 recombinant protein products, allowing them to bind while other proteins in the cell-free extract flow through the column. After loading the column was washed to baseline with PBS containing an additional 350mM NaCl. The IgG-Fc tagged proteins were eluted at low pH, either with 0.5M acetic acid or with a decreasing pH gradient of 0.1M citric acid and 0.2M disodium phosphate buffers. Tris base or disodium phosphate was added to the eluted protein to avoid prolonged exposure to low pH conditions.

The pooled protein was diafiltered into PBS or HEPES buffer and derivitized with 1 mM iodoacetamide to protect the exposed sulfhydryl group on the free cysteine near the hinge region of each Fc domain. This prevents disulfide mediated aggregation of proteins. A 6 ft² Millipore spiral wound ultrafiltration membrane with nominal 30 kiloDalton cutoff was used to perform the buffer exchange. The total protein was determined by UV absorbance at 280 nm using the diafiltration buffer as a blank. The relative amounts of heterodimer and two homodimer proteins were determined by SDS PAGE gel electrophoresis using a 6% Tris-Glycine gel (Novex). Gels were Coomassie-stained then transferred into destain solution overnight. A Shimadzu scanning densitometer was used to determine the relative intensity of the individual protein bands on the SDS PAGE gel. The peak area ratios are used to compute the fraction of heterodimer and each of the homodimers in the column pool fractions.

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Immobilized Metal Affinity Chromatographic Purification

The six histidine residues on the C-terminus of the GP130-Fc-His6 fusion protein provides an excellent molecular handle for separation of the heterodimeric IL6 antagonist from the two homodimers. The imidazole group on each of the C-terminal histidines of the GP130-Fc-His6 moiety has a strong binding constant with several divalent metals, including copper, nickel, zinc, cobalt, iron and calcium. Since the IL6Rα-Fc homodimer has no C-terminal histidine residues, it clearly has the lowest affinity. The IL6Rα-Fc-GP130-Fc-His6 heterodimer has a single stand set six histidines giving it greater affinity for the metal, while the GP130-Fc-His6 homodimer has two sets of six histidines each giving it the highest affinity of the three IgG tagged proteins to the metal affinity column. Selective elution of the three proteins with increasing amounts of imidazole in the elution buffer therefore elutes the proteins in the following order:

- 1. IL6Rα-Fc homodimer
- 2. IL6Rα-Fc-GP130-Fc-His heterodimer
- 20 3. GP130-Fc-His homodimer

A 26 mm diameter column containing 100 mL of Pharmacia Chelating Sepharose Fast Flow was saturated with a solution of nickel sulfate until a significant green color is observed in the column eluate. The column is then washed with several column volumes of deionized water, then equilibrated with 50 mM HEPES, 40mM imidazole, pH 8.0. The binding of imidazole to the immobilized nickel results in a green to blue color change. Imidazole was added to the protein load to a final concentration of 40mM. Addition of imidazole to the protein load reduces the binding of IL6R α -Fc homodimer, increasing the surface area available for the remaining two species. After loading, the column was washed with

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several column volumes of 50 mM HEPES, 80mM imidazole, pH 8.0 until a steady baseline was reestablished. The heterodimer was selectively eluted with 50 mM HEPES, 150mM imidazole, pH 8.0 over several column volumes. The protein fractions were pooled and diafiltered into PBS as described in the section above.

EXAMPLE 4. ALTERNATIVE METHODS OF CONSTRUCTING LIGAND TRAPS

- As described above, receptor activation by CNTF, and analogously by IL-6 10 and IL-11, follows an ordered sequence of binding events (Figure 6). The cytokine initially binds to its cognate $R\alpha$ with low affinity (Kd = 3 to 10 nM); this is a required step - cells which do not express the cognate Rα do not respond to the cognate cytokine. The cytokine•Rα complex associates with the first signal transducing component, gp130, to form a high affinity 15 complex (Kd in the order of 10 pM for the CNTF-CNTFRa•gp130 complex). This complex does not transduce signal, as it is the dimerization of the signal transducing components that brings about signaling (Stahl and Yancopoulos, J. Neurobiology 25: 1454-1466 (1994); Stahl et al., Science 267:1349-1353 (1995); Davis et al., Science 260:1805-1808 (1993); Stahl et al., 20 Science 263:92-95 (1994); Murakami, et al. Science 260:1808-1810 (1993). At least in the case of IL-6, the cytokine•Rα•signal transducer heterotrimeric complex subsequently associates with another like complex, to form a hexameric complex (Figure 6) (Ward et al., J. Biol. Chem. 269:23286-23289 (1994). The resulting dimerization of the signal transducers - gp130 in the 25 case of IL-6 (Murakami et al., Science 260:1808-1810 (1993) and IL-11, gp130 and LIFR in the case of CNTF (Davis et al., Science 260:1805-1808 (1993) brings about signal transduction.
- 30 The initial heterodimeric molecules made comprised a soluble $R\alpha$ component linked to the extracellular domain of gp130. These molecules

were shown to mimic the high affinity cytokine•Rα•gp130 complex and behave as a high affinity antagonist of their cognate cytokine (Figure 7). To make these molecules, the extracellular domain of gp130 was paired with the extracellular domain of the α -receptor components for IL-6 and CNTF, IL-6R α and CNTFR α respectively. To link the R α with the extracellular 5 domain of gp130, the soluble Rα-components and gp130 were fused to the Fc portion of human IgG1 to produce R α -Fc and gp130-Fc respectively. The Fc domain was chosen primarily but not solely because it naturally forms disulfide-linked dimers. Heterodimeric molecules comprising Rα-10 Fc•gp130-Fc were expressed, purified and shown to behave as highly potent antagonists of their cognate ligand. Furthermore, these molecules were found to be highly specific for their cognate cytokine since it is the choice of the α-receptor component which specifies which cytokine is bound and trapped (there is no measurable binding of the cytokine to

Here we describe an extension of this technology which allows the engineering of different heteromeric soluble receptor ligand traps which by virtue of their design may have additional beneficial characteristics such as stability, Fc-receptor-mediated clearance, or reduced effector functions (such as complement fixation). Furthermore, the technology described should prove suitable for the engineering of any heteromeric protein in mammalian or other suitable protein expression systems, including but not limited to heteromeric molecules which employ receptors, ligands, and catalytic components such as enzymes or catalytic antibodies.

MATERIALS AND METHODS

gp130 in the absence of the appropriate $R\alpha$).

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Genetic engineering of heteromeric immunoglobulin heavy/light chain soluble receptor-based ligand traps for IL-6.

The IL-6 traps described here were engineered using human gp130, human IL-6 α -receptor (IL-6R α), the constant region of the heavy chains (C γ) of human IgG1 (Cy1) (Lewis et al., Journal of Immunology 151:2829-2838 (1993) or IgG4 (Cy4) with or without a join-region (J), and the constant regions of kappa (κ) and lambda (λ) (Cheung, et al., Journal of Virology 5 66:6714-6720 (1992) light chains of human immunoglobulin (Ig), also with or without a different j-peptide (j). This design takes advantage of the natural ability of the Cy domain to heterodimerize with κ or λ light chains. The heterodimerization of Cy with the light chain occurs between the CH1 domain of Cy and the constant region of the light chain (CL), and is 10 stabilized by covalent linking of the two domains via a single disulfide bridge. We reasoned that, like the Fc domain of human IgG1, the combination of Cy with CL could be used to produce disulfide linked heteromeric proteins comprised of the extracellular domain of gp130 on one chain and the extracellular domain of IL-6R α on the other chain. Like 15 their Fc-based counterparts, such proteins were postulated to be high affinity ligand traps for IL-6 and as a result to inhibit the interaction of IL-6 with the native receptor on IL-6-responsive cells, thus functioning as IL-6 antagonists. Furthermore, constructs employing the full length Cy region would, much like antibodies, form homodimers of the Cy chain, giving 20 rise to antibody-like molecules comprising of two "light chains" and two "heavy chains" (Figure 8). The potential advantage of this design is that it may more closely mimic the IL-6•IL-6Rα•gp130 complex and may display a higher affinity for the ligand than comparable single heterodimers. An additional design is incorporated by using truncated versions of Cy, 25 comprised only of the CH1 domain. These will form heterodimeric molecules with receptor-κ fusion proteins, and will thus resemble the Fab fragment of antibodies.

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All the soluble receptor-Ig chimeric genes may be engineered in plasmid vectors including, but not limited to, vectors suitable for mammalian expression (COS monkey kidney cells, Chinese Hamster Ovary cells [CHO], and ras-transformed fibroblasts [MG-ras]) and include a Kozak sequence (CGC CGC CAC CAT GGT G) at the beginning of each chimeric gene for efficient translation. Engineering was performed using standard genetic engineering methodology. Each construct was verified by DNA sequencing, mammalian expression followed by western blotting with suitable antibodies, biophysical assays that determine ligand binding and dissociation, and by growth inhibition assays (XG-1, as described later). Since the domains utilized to engineer these chimeric proteins are flanked by appropriate restriction sites, it is possible to use these domains to engineer other chimeric proteins, including chimeras employing the extracellular domains of the receptors for factors such as IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, LIF, IL-11, IL-15, IFNγ, TGFβ, and others. The amino acid coordinates for each component utilized in making the IL-6 traps are listed below (Note: numbering starts with the initiating methionine as #1; long sequences are listed using the single letter code for the twenty amino acids):

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- (a) Constructs employing human gp130:
- (i) **gp130-C**γ**1** was engineered by fusing in frame the extracellular domain of gp130 (amino acids 1 to 619) to a Ser-Gly bridge, followed by the 330 amino acids which comprise Cγ1 and a termination codon (Figure 9).
- 25 (ii) **gp130-J-Cγ1** was engineered in the same manner as gp130-Cγ1 except that a J-peptide (amino acid sequence: GQGTLVTVSS) was inserted between the Ser-Gly bridge and the sequence of Cγ1 (see Figure 9).
 - (iii) **gp130**Δ3**fibro-C**γ1 was engineered by fusing in frame the extracellular domain of gp130 without its three fibronectin-like domains (Figure 10).
- 30 The remaining part of this chimeric protein is identical to gp130-Cγ1.

(iv) gp130-J-CH1 was engineered in a manner identical for that described for gp130-Cγ1, except that in place of the Cγ1 region only the CH1 part of Cγ1 has been used (Figure 11). The C-terminal domain of this construct includes the part of the hinge that contains the cysteine residue responsible for heterodimerization of the heavy chain of IgG with a light chain. The part of the hinge that contains the two cysteines involved in Cγ1 homodimerization has been deleted along with the CH2 and CH3 domains.

- (v) gp130-Cγ4 was engineered in a manner identical to that described for gp130-Cγ1, except that Cγ4 was used in place of Cγ1 (Figure 12). In addition, an RsrII DNA restriction site was engineered at the hinge region of the Cγ4 domain by introducing two silent base mutations. The RsrsII site allows for other desired genetic engineering manipulations, such as the construction of the CH1 equivalent of gp130-Cγ4.
- 15 (vi) **gp130**-κ was engineered in a manner identical to that described for gp130-Cγ1, except that the constant region of the κ light chain of human Ig was used in place of Cγ1 (Figure 13).
 - (vi) **gp130-J-** κ was engineered in a manner identical to that described for gp130-J- κ , except that a j-peptide (amino acid sequence: TFGQGTKVEIK) was inserted between the Ser-Gly bridge and the κ -region.
 - (viii) **gp130-** λ was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the λ light chain (Cheung, et al., Journal of Virology 66:6714-6720 (1992) of human Ig was used in place of C γ 1 (Figure 14).

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- (b) Constructs employing human IL-6Rα:
- (i) IL6R α -C γ 1 was engineered by fusing in frame amino acids 1 to 358 of IL-6R α (Yamasaki et al., Science 241:825-828 (1988), which comprise the

extracellular domain of IL-6R α (Figure 15), to an Ala-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon.

- (ii) **IL6R** α - κ was engineered as described for IL6R α -C γ 1, except that the κ -domain (Figure 13) utilized for gp130- κ was used in place of C γ 1.
- 5 (iii) **IL6R**α-**j**- κ was engineered as described for IL6Rα- κ except that the j-peptide described for gp130-j- κ was placed between the Ala-Gly bridge and the κ -domain.
- (iv) Three additional constructs, IL6Rα313-Cγ1, IL6Rα313-κ, and IL6Rα313-j-κ, were engineered as using a truncated form of IL-6Rα comprised of
 10 amino acids 1 to 313 (Figure 16). Each of these constructs were made by fusing in frame IL6Rα313 with a Thr-Gly bridge followed by the Cγ1, κ-, and j-κ-domains described above. These constructs were engineered in order to complement the gp130Δ3fibro-derived constructs.

15 Expression and purification of ligand traps

To produce covalently linked heterodimers of soluble gp130 and soluble IL-6Rα, gp130-Ig chimeric proteins were co-expressed with appropriate IL-6Rα-Ig chimeric proteins in complementing pairs. Co-expression was achieved by co-transfecting the corresponding expression vectors into suitable mammalian cell lines, either stably or transiently. The resulting disulfide-linked heterodimers were purified from conditioned media by several different methods, including but not limited to affinity chromatography on immobilized Protein A or Protein G, ligand-based affinity chromatography, ion exchange, and gel filtration.

An example of the type of methods used for purification of a heavy/light receptor fusion protein is as follows: gp130-Cγ1•IL-6Rα-κ was expressed in COS cells by co-transfecting two different vectors, encoding gp130-Cγ1 and

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IL-6Rα-κ respectively. Serum-free conditioned media (400 ml) were collected two days post-transfection and Cγ1-bearing proteins were purified by affinity chromatography over a 1ml Protein A Sepharose (Pharmacia). The material generated in this step was further purified by a second affinity chromatography step over a 1 ml NHS-activated Sepharose (Pharmacia) which was derivatized with recombinant human IL-6, in order to remove gp130-Cγ1 dimer from gp130-Cγ1•IL-6Rα-κ complexes (the gp130-Cγ1 dimer does not bind IL-6). Proteins generated by this method were more than 90% pure, as evidenced by SDS-PAGE followed by silver-staining (Figure 17). Similar protocols have been employed successfully towards the purification of other heavy/light receptor heterodimers.

RESULTS

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15 <u>Biological activity of immunoglobulin heavy/light chain receptor fusion</u> antagonists

The purified ligand traps were tested for their ability to bind IL-6 in a variety of different assays. For example, the dissociation rate of IL-6 bound to the ligand trap was measured in parallel with the dissociation rate of IL-20 6 from the anti-IL-6 monoclonal neutralizing antibody B-E8 [Brochier, et al., Int. J. Immunopharmacology 17:41-48 (1995), and references within]. An example of this type of experiment is shown in Figure 18. In this experiment 20 pM 125 I-IL-6 (1000 μ Ci/mmol; Amersham) was preincubated with 500 pM of either gp130-Cγ1•IL-6Rα-κ or mAb B-E8 for 20 25 hours. At this point a 1000-fold excess (20 nM) of "cold" IL-6 was added. Periodically, aliquots of the reaction were removed, the ligand trap or B-E8 were precipitated with Protein G-Sepharose, and the number of cpm of 125I-IL-6 that remained bound was determined. Clearly, the dissociation rate of human ¹²⁵I-IL6 from the ligand trap was very slow - after three 30 days, approximately 75% of the initial counts were still bound to the ligand

trap. In contrast, less than 5% of the counts remained associated with the antibody after three days. This result demonstrates that the dissociation rate of the ligand from these ligand traps is very slow.

5 In a different set of experiments the ability of the ligand traps to multimerize in the presence of ligand was tested. An example-of this is shown in Figure 19. IL-6-induced association of gp130-Fc•IL-6Rα-Fc with gp130-CH1•IL-6Rα-κ was determined by testing whether gp130-CH1•IL- $6R\alpha$ -κ, which does not by itself bind Protein A, could be precipitated by 10 Protein A-Sepharose in the presence of gp130-Fc•IL-6Rα-Fc in an IL-6depended manner (Figure 9). Precipitation of gp130-CH1•IL-6Rα-κ by Protein A-Sepharose was determined by western blotting with an antikappa specific HRP conjugate, which does not detect gp130-Fc•IL-6Rα-Fc. gp130-CH1•IL-6Rα-κ could be precipitated by Protein A-Sepharose only 15 when both gp130-Fc•IL-6Rα-Fc and IL-6 were present. This result conclusively indicates that IL-6 can induce ligand trap multimerization, and further indicate that the ligand trap can mimic the hexameric cytokine•Rα•signal transducer complex (Figure 1). Ligand-induced multimerization may play a significant role in the clearance of 20 cytokine•ligand trap complexes in vivo.

The biological activity of the different ligand traps may be further tested in assays which measure ligand-depended cell proliferation. Several cell proliferation assays exist for IL-6 and they employ cell lines such as B9, CESS, or XG-1. An example of this type of assay using the XG-1 cell line is presented below: XG-1 is a cell line derived from a human multiple myeloma (Zhang, et al., Blood 83:3654-3663 (1994). XG-1 depends on exogenously supplied human IL-6 for survival and proliferation. The EC50 of IL-6 for the XG-1 line is approximately 50 pmoles/ml. The ability of several different IL-6 traps to block IL-6-depended proliferation of XG-1

cells was tested by incubating increasing amounts of purified ligand traps with 50 pg/ml IL-6 in XG-1 cultures. The ligand traps which were tested had been expressed and purified by methods similar to those described above. All of the ligand traps tested were found to inhibit IL-6-dependent proliferation of XG-1 in a dose dependent manner (Figure 20). Of the five different traps tested gp130-Cγ1•IL-6Rα-κ was the most active and essentially display the same neutralizing activity towards IL-6 as the antibody B-E8. As little as a 10-fold molar excess of either gp130-Cγ1•IL-6Rα-κ or B-E8 completely blocked the activity of IL- 6 (a reading of A570-650 = 0.3 AU corresponds to no proliferation of the XG-1 cells). At a 100fold molar excess all of the ligand traps tested completely blocked the activity of IL-6. This observed inhibition is highly selective as neither a gp130-Fc•CNTFRα-Fc ligand trap which blocks CNTF activity, nor gp130-Fc homodimer exhibit any blocking activity towards IL-6 even when used at a 1000-fold molar excess over IL-6 (data not shown). This data demonstrates that the heteromeric immunoglobulin heavy/light chain receptor-based ligand traps function as selective high affinity antagonists of their cognate ligand.

20 EXAMPLE 5 - CLONING OF FUSION POLYPEPTIDE COMPONENTS

The extracellular domains of the human cytokine receptors were obtained by standard PCR techniques using tissue cDNAs (CLONTECH), cloned into the expression vector, pMT21 (Genetics Institute, Inc.), and the sequences were sequenced by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). For the IL-4Rα, nucleotides 241 through 868 (corresponding to the amino acids 24-231) from the Genbank sequence, X52425, were cloned. For the IL-2Rγ, nucleotides 15 through 776 (corresponding to amino acids 1-233) from the Genbank sequence, D11086, were cloned. For the IL-6Rα, nucleotides 52 through 1044 (corresponding

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to the amino acids 1-331) from the Genbank sequence, X52425, were cloned. For gp130, nucleotides 322 through 2112 (corresponding to the amino acids 30-619) from the Genbank sequence, M57230, were cloned. For the IL-1RAcP, nucleotides 1 through 1074 (corresponding to the amino acids 1-358) from the Genbank sequence, AB006357, were cloned. For the IL-1RI, nucleotides 55 through 999 (corresponding to the amino acids 19-333) from the Genbank sequence, X16896, were cloned.

EXAMPLE 6 - PRODUCTION OF FUSION POLYPEPTIDES (CYTOKINE TRAPS)

The nucleotide sequences encoding the cytokine traps were constructed from the individual cloned DNAs (described *supra*) by standard cloning and PCR techniques. In each case, the sequences were constructed in frame such that the sequence encoding the first fusion polypeptide component was fused to the sequence encoding the second fusion polypeptide component followed by an Fc domain (hinge, CH2 and CH3 region of human IgG1) as the multimerizing component. In some cases extra nucleotides were inserted in frame between sequences encoding the first and second fusion polypeptide components to add a linker region between the two components (See Figure 21A - Figure 21D - trap 424; Figure 24A - Figure 24F - trap 412; and Figure 26A - Figure 26E - trap 569).

For the IL-4 traps, 424 (Figure 21A - Figure 21D), 603 (Figure 22A - Figure 22D) and 622 (Figure 23A - Figure 23D), the IL-2Rγ component is 5′, followed by the IL4Rα component and then the Fc component. For the IL-6 traps, 412 (Figure 24A - Figure 24F) and 616 (Figure 25A - Figure 25F), the IL-6Rα component is 5′ followed by the gp130 component and then the Fc domain. For the IL-1 trap 569 (Figure 26A - Figure 26E), the IL-1RAcP component is 5′ followed by the IL-1RI component and then the Fc domain. The final constructs were cloned into the mammalian expression vector pCDNA3.1 (STRATAGENE).

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In the 569 sequence (Figure 26A - Figure 26E), nucleotides 1-1074 encode the IL1RAcP component, nucleotides 1075 -1098 encode a linker region, nucleotides 1099-2043 encode the IL1RI component and nucleotides 2044-2730 encode the Fc domain.

In the 412 sequence (Figure 24A - Figure 24F), nucleotides 1-993 encode the IL6Rα component, nucleotides 994-1023 encode a linker region, nucleotides 1024-2814 encode the gp130 component and nucleotides 2815-3504 encode the Fc domain.

In the 616 sequence (Figure 25A - Figure 25F), nucleotides 1-993 encode the IL6Rα component, nucleotides 994-2784 encode the gp130 component and nucleotides 2785-3474 encode the Fc domain.

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In the 424 (Figure 21A - Figure 21D) and 622 (Figure 23A - Figure 23D) sequences, nucleotides 1-762 encode the IL2R γ component, nucleotides 763-771 encode a linker region, nucleotides 772-1395 encode the IL4R α component and nucleotides 1396-2082 encode the Fc domain.

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Finally, in the 603 sequence (Figure 22A - Figure 22D), nucleotides 1-762 encode the IL2R γ component, nucleotides 763-1386 encode the IL4R α component and nucleotides 1387-2073 encode the Fc domain.

DNA constructs were either transiently transfected into COS cells or stably transfected into CHO cells by standard techniques well known to one of skill in the art. Supernatants were collected and purified by Protein A affinity chromatography and size exclusion chromatography by standard techniques. (See for example Harlow and Lane, Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

EXAMPLE 7: IL-4 BIOASSAY PROTOCOL USING TF-1 (ATCC) CELLS.

Reagents and Equipment Needed

5 MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128) Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca+2, Mg+2.

10 Sterile filter and store aliquoted at -20°C

Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml d H_2 0, 50 ml Dimethyl Formamide, and 850 μ l concentrated HCl.

Filter sterilize with a 0.45µm filter unit.

Store at room temperature

TF-1 cell Growth Medium:

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RPMI 1640, 10% FBS, Pen/Strep, 2mM L-glutamine

Other:

25 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon #3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100µl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

Assay Protocol

A. Preparation of Assay plates

Prepare sterile 96 well tissue culture plates to contain 50μl of growth medium per well with various concentrations of IL-4 and 10nM IL-4 antagonist. This can be done by preparing a working dilution of IL-4 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-4. Add 25μl of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25μl of growth medium without IL-4 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25μl to a triplicate set of IL-4 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H.

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- 2. As a positive control, leave one set with no antagonist. These wells will contain IL-4 and media only.
- 3. Incubate the plate for 1-2 hours at 37°C in a humidified 5% CO₂
 20 incubator before preparing cells to be used for assay.

B. Preparation of Cells

- 4. Wash cells twice by centrifugation in assay medium free of growth25 factor.
 - 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.
- 30 6. Dispense 50μl of the cell suspension (40,000 cells) into all wells of the plates. Total volume should now be 100μl/well.

7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

C. Color Development

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- 8. After incubating for 68 hours, add 15µl of the MTT dye solution to each well.
- 9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

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- 10. After 4 hours, add 100µl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.
- 15 11. Record the absorbance at 570/650nm.

RESULTS

Figure 27 shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.

Figure 28 shows that the IL-4 trap designated 4SC375 shows antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated

4SC424 which is a fusion polypeptide of IL-2Rγ-IL4Rα-FcΔC1 having the IL-2Rγ component flush with the IL-4Rα component.

EXAMPLE 8: IL-6 BIOASSAY PROTOCOL USING XG-1 CELLS

30 Reagents and Equipment Needed

MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca^{+2} , Mg^{+2} .

Sterile filter and store aliquoted at -20°C

Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml dH_20 , 50 ml Dimethyl Formamide, and 850 μ l concentrated HCl.

Filter sterilize with at 0.45µm filter unit.

Store at room temperature

15 Assay Medium:

RPMI 1640, 10%FBS, Pen/Strep, 2mM L-glutamine, 50µM mercaptoethanol.

20 Other:

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0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100µl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

Assay Protocol

A. Preparation of Assay plates

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1. Prepare sterile 96 well tissue culture plates to contain 50µl of growth medium per well with various concentrations of IL-6 and 10nM IL-6 antagonist. This can be done by preparing a working dilution of IL-6 that is

4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-6. Add 25µl of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25µl of growth medium without IL-6 to row H.

- Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25µl to a triplicate set of IL-6 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H. A typical IL-6 titration starts at 200ng/ml down to 3.1ng/ml.
- 10 2. As a positive control, leave one set with no antagonist. These wells contain IL-6 and media in place of antagonist.
 - 3. Incubate the plate 1-2 hours at 37oC in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

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B. Preparation of Cells

4. Wash cells twice by centrifugation (5 min at 1000RPM) in assay medium free of growth factor.

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- 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of $8 \times 10^5/\text{ml}$ in assay medium.
- 6. Dispense 50µl of the cell suspension (40000 cells) into all wells of the plates. Total volume should now be 100µl/well.
 - 7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

30 <u>C. Color Development</u>

8. At 68 hours add $15\mu l$ of the dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

10. After 4 hours, add 100µl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

11. Record the absorbance at 570/650nm.

RESULTS

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Figure 29 shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc Δ C1) described in Figure 24A - Figure 24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

15 EXAMPLE 9: MRC5 BIOASSAY FOR IL1 TRAPS

MRC5 human lung fibroblast cells respond to IL-1 by secreting IL-6 and thus were utilized to assay the ability of IL-1 traps to block the IL-1-dependent production of IL-6. IL1 Trap 1SC569 (Figure 26A - Figure 26E) was tested against IL-1-RI.Fc which is the extracellular domain of the IL-1 Type I receptor fused to an Fc domain.

MRC5 cells are suspended at 1×10^5 cells per ml in medium and 0.1 ml of cells are plated (10,000 cells per well) into the wells of a 96 well tissue culture plate. Plates are incubated for 24 hours at 37°C in a humidified 5% CO_2 incubator.

IL-1 trap and recombinant human IL-1 at varying doses are pre-incubated in a 96 well tissue culture dish and incubated for 2 hours at 37°C. 0.1 ml of this mixture is then added to the 96 well plate containing the MRC5 cells such that the final concentration of IL-1 Trap is 10nM and the final

concentrations of the IL-1 ranges from 2.4 pM to 5nM. Control wells contain trap alone or nothing.

Plates are then incubated at 37°C for 24 hours in a humidified 5% CO₂ incubator. Supernatant is collected and assayed for levels of IL-6 using R&D Systems Quantikine Immunoassay Kit according to the manufacturer's instructions.

RESULTS

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Figure 30 shows that the trap 569 (Figure 26A - Figure 26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1. At a concentration of 10nM, the trap 569 is able to block the production of IL-6 up to an IL-1 concentration of 3nM. In contrast, the IL-1RI.Fc is a much poorer antagonist of IL-1. It is only able to block the effects of IL-1 up to about 10-20 pM. Thus, the trap 569 is approximately 100x better at blocking IL-1 than IL1RI.Fc.

EXAMPLE 10 - CONSTRUCTION OF IL-13/IL-4 SINGLE CHAIN TRAPS

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1. To create the IL-13/IL-4 dual trap designated IL-4R α .IL-13R α 1.Fc, the human IL-4R α extracellular domain (corresponding to nucleotides #1-693 of Figure 31A - Figure 31G) and the human IL-13R α 1 extracellular domain (corresponding to nucleotides #700-1665 of Figure 31A - Figure 31G) were amplified by standard PCR techniques and ligated into an expression vector pMT21 which contained the human Fc sequence (corresponding to nucleotides #1671-2355 of Figure 31A - Figure 31G), thus creating a fusion protein consisting of the IL-4R α , IL-13R α 1, and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a two amino acid linker (corresponding to nucleotides #694-699 of Figure 31A - Figure 31G) with the amino acid sequence SerGly was constructed in frame

between the IL-4R α and the IL-13R α 1 and a two amino acid linker (corresponding to nucleotides #1666-1671 of Figure 31A - Figure 31G) with the amino acid sequence ThrGly was constructed in frame between the IL-13R α 1 and the Fc portion. All sequences were sequence-verified by standard techniques. The IL-4R α .IL-13R α 1.Fc coding sequence was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

2. To create the IL-13/IL-4 dual trap designated IL-13Rα1.IL-4Rα.Fc, the IL-10 13Rα1 extracellular domain (corresponding to nucleotides #1-1029 of Figure 32A - Figure 32G) and the human IL-4Rα (corresponding to nucleotides # 1060-1692 of Figure 32A - Figure 32G) were amplified by standard PCR techniques and ligated into the expression vector pIFE14. which contains the human Fc sequence (corresponding to nucleotides 15 #1699-2382 of Figure 32A - Figure 32G) to create a fusion protein consisting of the IL-13R α 1, IL-4R α , and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a ten amino acid linker with the amino acid sequence GlyAlaProSerGlyGlyGlyGlyArgPro (corresponding to nucleotide #1030-1059 of Figure 32A - Figure 32G) was 20 constructed in frame between the IL-13R α 1 and the IL-4R α and a two amino acid linker (corresponding to nucleotides #1693-1698 of Figure 32A -Figure 32G) with the amino acid sequence SerGly was constructed in frame between IL-4R α and the Fc portion. All sequences were sequence-verified using standard techniques. The coding sequence of IL-13Rα1.IL-4Rα.Fc was then subcloned into the expression vector pCDNA3.1 (Stratagene) 25 using standard molecular biology techniques.

EXAMPLE 11: EXPRESSION OF IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc

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Large scale (1L) cultures of the pCAE801 (the DNA vector construct encoding IL-4Rα.IL-13Rα1.Fc) and pCAE802 (the DNA plasmid construct encoding IL-13Rα1.IL-4Rα.Fc) in DH10B cells were grown overnight in LB + ampicillin and the plasmid DNA was extracted using a Qiagen Endofree Mega Kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of aliquots with BbsI, XmnI and NcoI restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

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Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4×10^6 cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 µg of pCAE801, or pCAE802, using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days.

After 3 days of incubation the media was removed from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and expressed protein was purified as described *infra*.

EXAMPLE 12: PURIFICATION OF IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc PROTEIN FROM CULTURE MEDIA

1. Purification of IL-4Rα.IL-13Rα1.Fc.

Human IL-4Rα.IL-13Rα1.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described supra. 5 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (y chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield ranged from 5.8 to 9.2 mg (average of 7.5 mg) per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche 10 Diagnostics Corp.) were dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 µm pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap® Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The 15 column was extensively washed with PBS buffer to remove nonspecifically bound proteins from the column. IL-4Ra.IL-13Ra1.Fc_was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, 20 pH 7.4 at 4°C. The recovery from Protein A purification was 6.8 mg (73%). IL-4Rα.IL-13Rα1.Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were 25 assessed from a Coomassie stained non-reduced and reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were conservatively pooled to reduce the amount of aggregated protein. The overall yield was 51% (4.4 mg) with a purity of 97% as judged by SDS-PAGE. Purified IL-4Rα.IL-13Rα1.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-30 12% Bis-Tris), analytical size exclusion chromatography (Tosohaas

TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

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2. Purification of IL-13Rα1.IL-4Rα.Fc

Human IL-13Rα1.IL-4Rα.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described supra. Expression of the secreted protein was determined by a sandwich ELISA 10 using goat anti-hIgG (y chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield was 8.8 mg per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche Diagnostics Corp.) were dissolved into the media (1 tablet/L). The conditioned media was sterile 15 filtered (0.22 µm pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap® Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The column was extensively washed with PBS buffer to remove nonspecifically bound proteins from the column. IL-13Rα1.IL-20 4Rα.Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, pH 7.4 at 4 °C. The recovery from Protein A purification was 3.8 mg (43%). IL-13Rlpha1.IL-4Rlpha.Fc was further purified by size exclusion 25 chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were assessed from a Coomassie stained non-reduced and reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were 30

conservatively pooled to reduce the amount of aggregated protein. The overall yield was 17% (1.5 mg) with a purity of 95% as judged by SDS-PAGE. Purified IL-13Rα1.IL-4Rα.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-12% Bis-Tris), analytical size exclusion chromatography (Tosohaas TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4Rα (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

10 EXAMPLE 13: BLOCKING OF IL-4 AND IL-13 BY IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc

Materials and Methods

TF1 Bioassay. TF1 cells were maintained in growth media (10ng/ml GM-CSF, RPMI 1640, 10% FBS, L-glutamine, Penicillin, Streptomycin). For the bioassay, cells were washed 2 times in assay media (as above but without GM-CSF) and then plated at 2 x 10⁵ cells in 50μl of assay media. The purified IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc proteins were diluted into assay media at a concentration of 40nM. 25ul of each of the traps was added to the cells. Either IL-13 or IL-4 were diluted to 40nM in assay media and then 2-fold dilution series in assay media were made. 25μl of either IL-13 or IL-4 was then added to the wells containing the cells and the traps. Cells were then incubated at 37°C, 5% CO₂ for ~70 hrs. The extent of TF1 cell proliferation was measured by the MTS assay according to the manufacturer's protocol (Promega, Inc.).

RESULTS

The ability of the IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc traps to block both human IL-13 and human IL-4 activity was measured in the TF1

bioassay described *supra*. IL-13 stimulates proliferation of TF1 cells, with half-maximal growth at a concentration of 0.2nM. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM (Figure 33). At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%. TF1 cells are more sensitive to IL-4, which stimulates their proliferation with half-maximal growth at ~0.02nM. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM (Figure 34). At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%. These results show that both IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc can block the ability of both IL-13 and IL-4 to stimulate cellular responses.

EXAMPLE 14: BLOCKING OF INJECTED IL-1 BY IL-1 TRAP IN VIVO

IL-1 is a pro-inflammatory cytokine. Systemic administration of IL-1 has been shown to elicit acute responses in animals, including transient hyperglycemia, hypoinsulinemia, fever, anorexia, and increased serum levels of interleukin-6 (IL-6) (Reimers, 1998). Since mice are responsive to both murine and human IL-1, human IL-1 can be used and *in vivo* binding effects of human specific IL-1 antagonists can be evaluated. This acute mouse model was used to determine the ability of a human IL-1 trap to antagonize the *in vivo* effects of exogenously administered human IL-1. This provides a rapid indication of *in vivo* efficacy of the human IL-1 trap and can be used as an assay to help molecule selection.

Experimental Design:

Mice were given subcutaneous injections of human IL-1 (0.3 μg/kg).

Twenty-four hours prior to human IL-1 injection, the animals were pretreated with either vehicle or 150-fold molar excess of human IL-1 trap (0.54 mg/kg). Two hours prior to sacrifice (26 hrs), the mice were given a

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second injection of human IL-1 (0.3 µg/kg). Blood samples were collected at various time points and sera were assayed for IL-6 levels.

RESULTS

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Exogenous administration of human IL-1 resulted a dramatic induction of serum IL-6 levels. At 150-fold molar excess, the human IL-1 trap completely blocked the IL-6 increase (Figure 35). Furthermore, the effects of the human IL-1 trap persisted for at least another 24 hours, preventing an IL-6 increase even when IL-1 was re-administered (Figure 35). Such long-lasting efficacy suggests that daily injection of an IL-1 trap may not be necessary for chronic applications.

EXAMPLE 15: EVALUATING THE ABILITY OF AN IL-4 TRAP TO

BLOCK THE PHYSIOLOGICAL RESPONSES TO HUMAN IL-4 IN

CYNOMOLOGUS MONKEYS.

Systemic administration of human IL-4 elicits systemic responses in Cynomologus monkeys (Gundel et al., 1996). Thus, the effectiveness of the IL-4 trap in blocking human IL-4 can be demonstrated by measuring these responses.

Experimental Design:

The experiment consisted of 3 parts: human IL-4 + vehicle (part 1), human IL-4 + IL-4 Trap (part 2), and human IL-4 + vehicle (part 3).

Human IL-4 (25 μg/kg) was injected subcutaneously twice daily for 4 days and IL-4 Trap (8 mg/kg) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16 and plasma was obtained to assay for the cytokine monocyte chemotactic protein 1 (MCP-1).

CD16 and MCP-1 are markers of IL-4-mediated inflammation in both humans and monkeys.

RESULTS

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In the presence of human IL-4, MCP-1 increased 2.5-fold and was significantly blocked by the IL-4 Trap (Figure 36A). Similarly, the decrease in the percent of CD16 positive lymphocytes in peripheral blood was attenuated by the IL-4 trap (Figure 36B). After a rest period, the monkeys were re-injected with human IL-4 and the responsiveness of the animals to human IL-4 was re-confirmed (Figures 36A and 36B), suggesting that inhibition of the MCP-1 and CD 16 responses is specifically mediated by the IL-4 trap.

15 EXAMPLE 16: THE EFFECTS OF IL-4 TRAP ON 1L-4-INDUCED IgE SECRETION.

It has been shown that injection of anti-mouse IgD antibody stimulates an IL-4-mediated IgE increase in normal mice. This model has been widely used to evaluate IL-4 antagonists, such as soluble IL-4 receptor and anti-IL-4 monoclonal antibodies (Sato et al., 1993). We decided to use this model to evaluate the ability if the IL-4 trap to block IL-4-mediated increases of IgE.

25 Experimental design:

BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups. Each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Serum was collected at various time points and assayed for IgE levels.

RESULTS

Treatment with the murine IL-4 trap or the mouse IL-4 antibody both significantly antagonized the IL-4-mediated IgE increase in this mouse model (Figure 37). This suggests that the murine IL-4 trap binds murine IL-4 and antagonizes physiological responses elicited by endogenous IL-4 *in vivo*.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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WE CLAIM:

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1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:

- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
- b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
- c) a nucleotide sequence encoding a third fusion polypeptide
 15 component comprising the amino acid sequence of a multimerizing component.
 - 2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component.
 - 3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component.

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4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor and cardiotrophin-1

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

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6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

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7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

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- The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF-β/BMP family selected from the group consisting of TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.
- 9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18 and MIF.
- 30 10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

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- 12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.
- 13. A composition capable of binding a cytokine to form a10 nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.
 - 14. The composition of claim 13, wherein the multimer is a dimer.
- 15 15. A vector which comprises the nucleic acid molecule of claim 1.
 - 16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

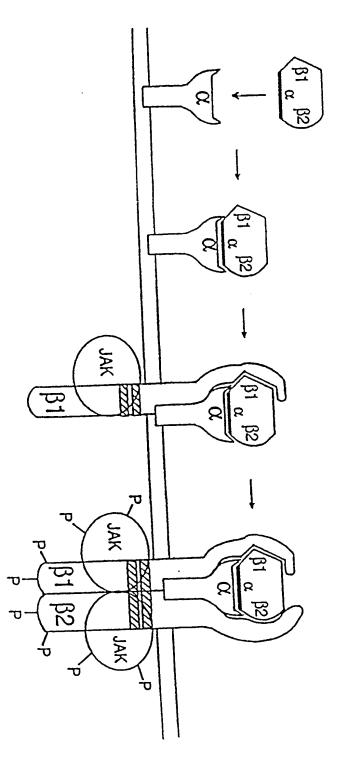
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- 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.
- 18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.
 - 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
- 30 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

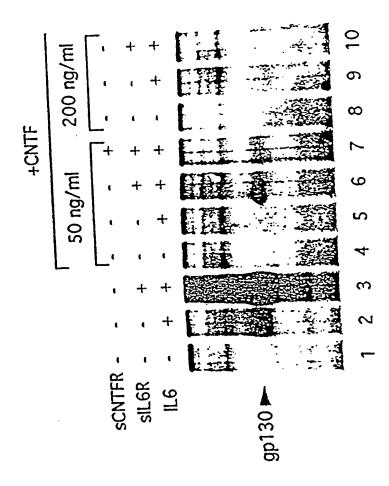
21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.

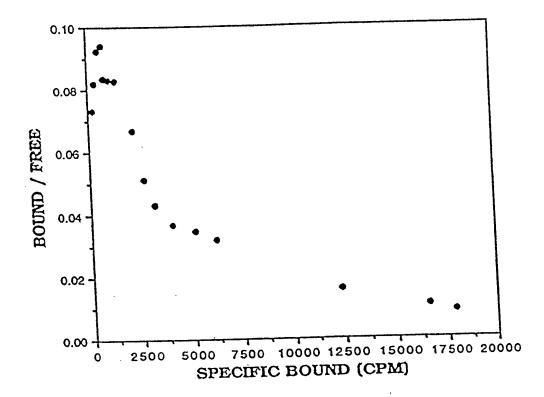
- 22. The host-vector system of claim 17, wherein the suitable host cell is a 293 cell.
 - 23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.
- 10 24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.
 - 25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions
- permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

1/ 구석 FIGURE 1



2/ 74 FIGURE 2





Amino acid sequence of human gp130-Fc-His6

Sequence Range:	1 to 861				
10	20	30	40	50 *	60 *
* MVTLQTWVVQALFIF	* LTTES TGELL	DPCGYISPESI	OVVOL HSNF	TAVCVLKEKCMI	YFHV
70	80	90	100	110	120
NANYIVWKTNHFTIP		* ASSVTFTDIA	* SLNIQ LTCN	ILTFGQLEQNV	YGITI
		150	160	170	180
130 * ISGLPPEKPKNLSC	140 *		* TNFTL KSE	* WATHKFADCKAK	RDTPT
ISGLPPEKPKNLSC			220	230	240
190	200	210	*	* PPHNLSVINSE	* ELSSIL
* SCTVDYSTVYFVNI	EVWVEA ENAL	GKVTSDHINFI		290	300
250	260	270 *	280	*	*
* KLTWTNPSIKSVII	LKYNIQ YRTH	CDASTWSQIPP		SETAÖDPV51.F	360
310	320	330 *	3 4 0	350 *	*
CMKEDGKGYWSDW:	SEEASGI TYE	DRPSKAPSFW	KIDPSH TQ	GYRTVQLVWKTI	PPFEAN
370	380	390	400	410 *	420 *
* GKILDYEVTLTRW	KSHLQNY TVN	ATKLTVNLTN	DRYLATL T	/RNLVGKSDAAV	LTIPACD
430	440	450	460 *	470 *	480 *
* FQATHPVMDLKAI	* FPKDNMLW VE	wttpresvkky	ILEWCVL S	DKAPCITDWQQE	DGTVHRT
490	500	510	520	530 *	540 *
YLRGNLAESKCY	* T.TTVTTVT AD	GPGSPESIKA	YLKQAPPS F	(GPTVRTKKVGKI	NEAVLEWD
550	560	570	580	590	600
QLPVDVQNGFI		* GNETAVNVDS	* SHTEYTLS	SLTSDTLYMVRM	AAYTDEGG
	620	630	640	650	660
610			t t *	LGGPSVFLFPP	KPKDTLMIS
KDGPEFTFTTP			700	710	720
670	680 *	690	*	* OVNSTYRVVSV	* LTVLHODWL
RTPEVTCVVVI	VSHEDPEVK I			OYNSTYRVVSV	780
730	740 *	750	760	*	

FIGURE 4 continued

NGKEYKCKVSNKALPAPIEK TISKAKGOPREPOVYTLPPS RDELTKNOVSLTCLVKGFYP

790 800 810 820 830 840

SDIAVEWESNGOPENNYKTT PPVLDSDGSFFLYSKLTVDK SRWOOGNVFSCSVMHEALHN

850 860 * *

HYTOKSLSLSPGKHHHHHH.

The amino acid sequence of human IL-6R α -Fc

Sequence Range: 1 to 594

Sequence Range:	1 60 234						
10	20	30	40	50 *	60 *		
* MVAVGCALLAALLA!	Y NPGAAL APRR	CPAQEVARGVI	TSLPG DSVT	LTCPGVEPEDN	WHVTA		
70	80	90	100	110	120		
* * * VLRKPAAGSHPSRWAGMGRR LLLRSVQLHDSGNYSCYRAG RPAGTVHLLVDVPPEEPQLS							
130	140	150	160	170	180 *		
* CFRKSPLSNVVCEW	* GPRSTP SLT	* rkavllvrkfQ1	NSPAED FQE	PCQYSQESQKF	SCQLAV		
190	200	210	220	230	240		
* PEGDSSFYIVSMCV	* 'ASSVGS KFS	* KTQTFQGCGIL	QPDPPA NIT	VTAVARNPRWL	SVTWQD		
250	260	270	280	290	300		
* PHSWNSSFYRLRFI	* ELRYRAE RSK	* TFTTWMVKDLQ	HHCVIH DAW	'SGLRHVVQLRA	QEEFGQ		
310	320	330	340	350	360		
* GEWSEWSPEAMGT	*	* \ENEVSTPMQAI	TTNKDD DN	* LLFRDSANATSI	PVQDAG		
370	380	390	400	410	420		
*† EPKSCDKTHTCPP	t * CPAPELL GG	* PSVFLFPPKPK	* DTLMISR TP	EVTCVVVDVSH	EDPEVKF		
430	440	450	460	470	480		
NWYVDGVEVHNA!	+	* STYRVVSVLTV	LHODWLN GK	* EYKCKVSNKAL	PAPIEKT		
490	500	510	520	530	540		
1SKAKGOPREPO	•	*	* LVKGFYPS_D	* TAVEWESNGOPI	* ENNYKTTP		
		570	580	590			
550 *	560	*	*	*	•		
PVLDSDGSFFLYSKLTVDKS RWOOGNVFSCSVMHEALHNH YTOKSLSLSPGK.							

FIGURE: 6

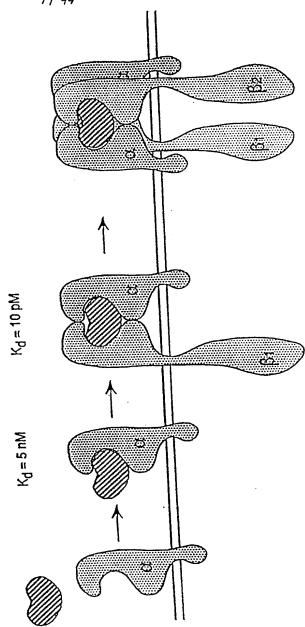
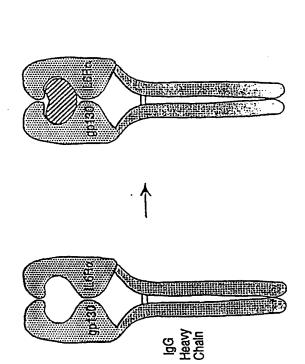
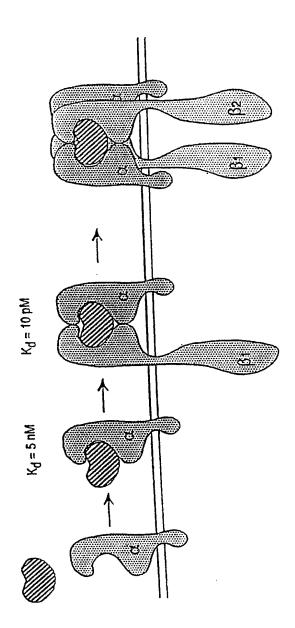
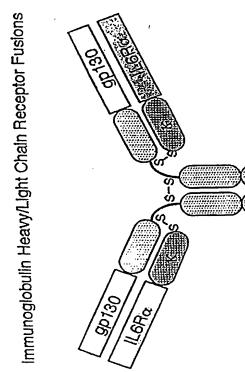


FIGURE 7
Heterodimeric Receptor-Based Ligand Trap





FIGURE



10/74 FIGURE 9

Amino acid sequence of gp130-Cyl

Sequence Range: 1 to 952 20 30 40 50 60 MVTLQTWVVQALFIFLTTES TGELLDPCGYISPESPVVQL HSNFTAVCVLKEKCMDYFHV NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTFGQLEQNVYGITI ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT 200 210 220 230 SCTVDYSTVYFVNIEVWVEA ENALGKVTSDHINFDPVYKV KPNPPHNLSVINSEELSSIL KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPFTEYVFRIR CMKEDGKGYWSDWSEEASGI TYEDRPSKAPSFWYKIDPSH TQGYRTVQLVWKTLPPFEAN GKILDYEVTLTRWKSHLQNY TVNATKLTVNLTNDRYLATL TVRNLVGKSDAAVLTIPACD FQATHPVMDLKAFPKDNMLW VEWTTPRESVKKYILEWCVL SDKAPCITDWQQEDGTVHRT 500 510 520 530 * * * YLRGNLAESKCYLITVTPVY ADGPGSPESIKAYLKQAPPS KGPTVRTKKVGKNEAVLEWD QLPVDVQNGFIRNYTIFYRT IIGNETAVNVDSSHTEYTLS SLTSDTLYMVRMAAYTDEGG KDGPEFTFTTPKFAQGEIES GASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTV 670 680 690 700 * * * SWNSGALTSGVHTFPAVLOS SGLYSLSSVVTVPSSSLGTO TYICNVNHKPSNTKVDKKVE 730 740 750 760 * * * PKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFN 11/74
FIGURE 9 continued

790 8L 810 820 8: 840

WYVDGVEVHNAKTKPREEOY NSTYRVVSVLTVLHODWLNG KEYKCKVSNKALPAPIEKTI

850 860 870 880 890 900

SKAKGOPREPOVYTLPPSRD ELTKNOVSLTCLVKGFYPSD LAVEWESNGOPENNYKTTPP

910 920 930 940 950

VLDSDGSFFLYSKLTVDKSR WOOGNVFSCSVMHEALHNHY TOKSLSLSPGK*

12/ 74 FIGURE: 10

Amino acid sequence or gp130\Delta3fibro

10 20 30 40 50 60 WYTLQTWVVQALFIFLTTES TGELLDPCGYISPESPVVQL HSNFTAVCVLKEKCMDYFHV

70 80 90 100 110 120 ***
NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTFGQLEQNVYGITI

130 140 150 160 170 180

ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT

190 200 210 220 230 240

SCTVDYSTVYFVNIEVWVEA ENALGKVTSDHINFDPVYKV KPNPPHNLSVINSEELSSIL

250 260 270 280 290 300 * * * *

KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPFTEYVFRIR

310 320 330

CMKEDGKGYWSDWSEEASGI TYEDRPSKAPSG

Sequence Range: 1 to 332

FIGURE 11

Amino acid sequence of J-CH1

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FIGURE 12

Amino acid sequence of C74

Sequence Range:	1 to 3	330			
10	20	30	40 *	50 *	60
SGASTKGPSVFPLAP	CSRST :	SESTAALGCLVKI	YFPEPVT	VSWNSGALTSGV	HTFPAVLQ
70 *	80	90 *	100	110	120
SSGLYSLSSVVTVPS	SSLGT	KTYTCNVDHKPSI	NTKVDKRV	ESKYGPPCPSCI	PAPEFLGGP
130	140	150 *	160 *	170	180 *
SVFLFPPKPKDTLMI	SRTPE	VTCVVVDVSQED	PEVQFNWY	VDGVEVHNAKT	KPREEQFNS
190	200	210	220	230	240
TYRVVSVLTVLHQD	VLNGKE	YKCKVSNKGLPS	SIEKTISK	AKGQPREPQVY	TLPPSQEEM
250 *	260 *	270 *	280	290 *	300 *
TKNQVSLTCLVKGF	YPSDIA	VEWESNGQPENN	YKTTPPVL	DSDGSFFLYSR	LTVDKSRWQ
310	320	330 *			
EGNVFSCSVMHEAL	нинуто	KSLSLSLGK*			

FIGURE 13

Amino acid sequence of κ -domain

Sequence Range: 1 to 108

10 20 30 40 50 60

SGTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQ

70 80 90 100

DSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVT KSFNRGEC*

FIGURE 14

Amino acid sequence of λ -domain:

Sequence Range: 1 to 107

10 20 30 40 50 60

SGPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSK

70 80 90 100

QSNNKYAASSYLSLTPEQWK SHRSYSCQVTHEGSTVEKTV APTECS*

17/ 74 FIGURE 15

Amino acid sequence of the soluble IL-6κα domain

Sequence Rang	ge: 1 to 36	0			
10	20	30	40	50 *	60 *
MVAVGCALLAALI	LAAPGAAL AF	RRCPAQEVARGV	LTSLPG DSV	TLTCPGVEPEDI	WHVTAN
70	80	90	100	110	120
VLRKPAAGSHPS	RWAGMGRR LI	LLRSVQLHDSGN	SCYRAG RPA	'GLAHFFADA bh	EEPQLS
130	140	150	160 *	170 *	180
CFRKSPLSNVVC	EWGPRSTP S	LTTKAVLLVRKF	QNSPAED FQ	EPCQYSQESQKE	SCOLVA
190	200	210	220	230	240
PEGDSSFYIVS	CVASSVGS K	FSKTQTFQGCGI	LQPDPPA NI	TVTAVARNPRW	LSVTWQD
250	260	270	280	290	300 *
PHSWNSSFYRL	RFELRYRAE I	RSKTFTTWMVKDI	QHHCVIH DA	WSGLRHVVQLR	AQEEFGQ
310	320	330	340	350	360 *
* GEWSEWSPEAM	GTPWTESRS	PPAENEVSTPMQ	ALTINKDD DI	NILFRDSANATS	SLPVQDAG

FIGURE 16

Amino acid sequence of the soluble IL-6kv313 domain

Sequence Rang	ge: 1 to 3	15			
10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAAL	LAAPGAAL /	APRRCPAQEVARG	VLTSLPG I	SVTLTCPGVEPE	WHVTAND
70	0.0	90	100	110	120
70	80	9 0	*	*	*
VLRKPAAGSHPS	RWAGMGRR	LLLRSVQLHDSG1	YSCYRAG 1	RPAGTVHLLVDVI	PEEPQLS
		450	160	170	180
130	140	150	100	*	*
* CFRKSPLSNVVC	* CEWGPRSTP	* SLTTKAVLLVRKI	FQNSPAED	FQEPCQYSQESQI	KFSCQLAV
400	200	210	220	230	240
190	200	210	*	*	*
PEGDSSFYIVS	MCVASSVGS	KFSKTQTFQGCG	ILQPDPPA	NITVTAVARNPR	WLSVTWQD
252	260	270	280	290	300
250	260	270	*	*	*
PHSWNSSFYRL	RFELRYRAE	RSKTFTTWMVKI	LQHHCVIH	DAWSGLRHVVQI	RAQEEFGQ
310					
GEWSEWSPEAM	CTTC ·				
GERGEROF BRE					

FIGURE 17

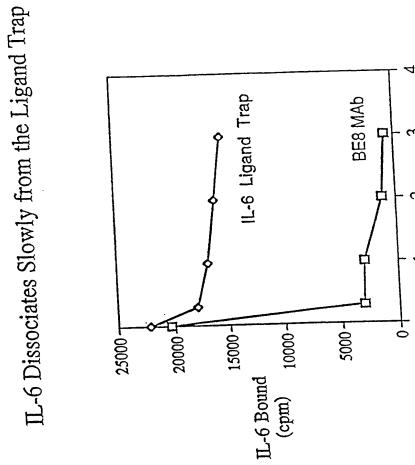
$$(gpx-C\gamma 1)_2 - \frac{1}{200} \frac{(gpx-C\gamma 1)_2 \cdot (6R\kappa)_2}{(gpx-C\gamma 1)_2 \cdot (6R\kappa)_2}$$

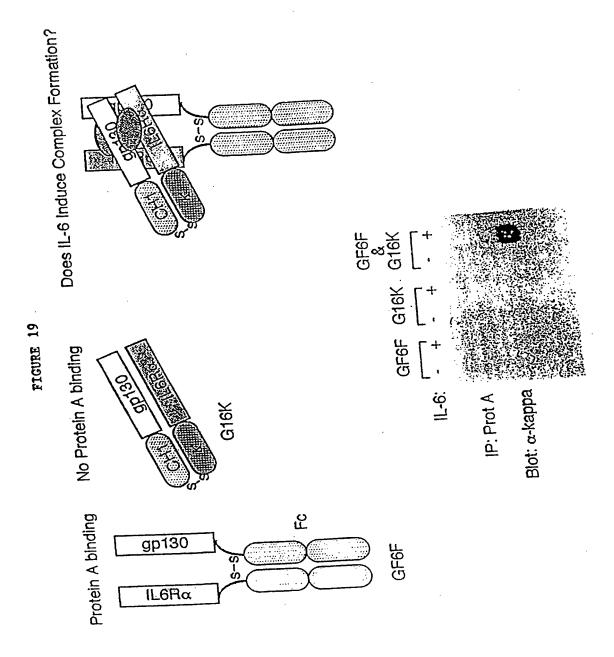
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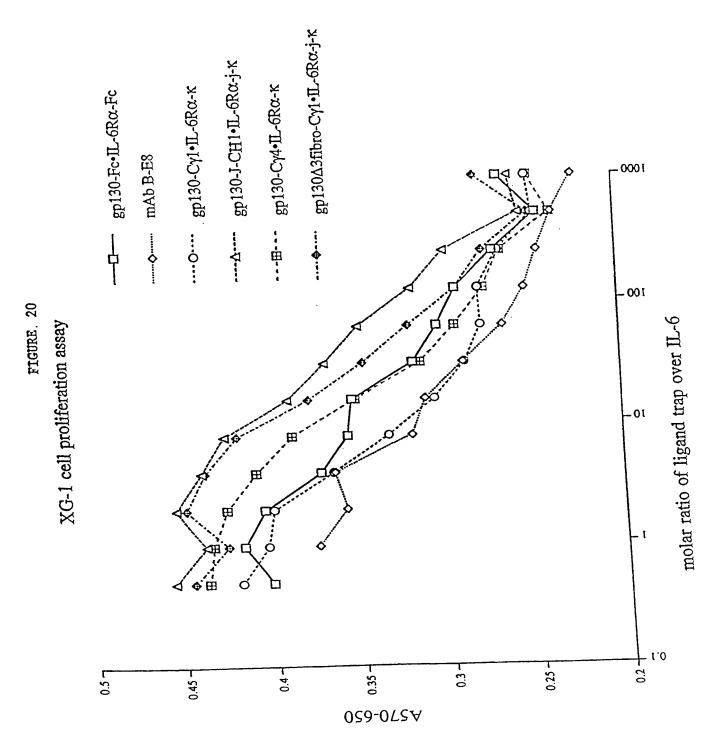
Days

FIGURE 18









10	20	30	40	*
* * * ATG GTG AAG CC Met Val Lys Pr	A TCA TTA CCA o Ser Leu Pro	TTC ACA TCC O	CTC TTA TTC CT Leu Leu Phe Le	G CAG CTG u Gln Leu>
50 6	50	70 * *	80 9 * *	0 * *
CCC CTG CTG GC Pro Leu Leu G	GA GTG GGG CTG Ly Val Gly Leu	AAC ACG ACA Asn Thr Thr	ATT CTG ACG CC Ile Leu Thr Pr	C AAT GGG O Asn Gly>
100	110	120	130	140
* * AAT GAA GAC A Asn Glu Asp T	CC ACA GCT GAT hr Thr Ala Asp	י ምጥር ጥጥር ርጥG	ACC ACT ATG CO	CC ACT GAC ro Thr Asp>
150	160	170	180	190
* * TCC CTC AGT G Ser Leu Ser V	* * * TT TCC ACT CT al Ser Thr Le	* * G CCC CTC CCA u Pro Leu Pro	GAG GTT CAG T Glu Val Gln C	GT TTT GTG ys Phe Val>
200	210	220	230	240
* * TTC AAT GTC (Phe Asn Val (* * GAG TAC ATG AA Glu Tyr Met As	T TGC ACT TGG n Cys Thr Trp	AAC AGC AGC T Asn Ser Ser S	CT GAG CCC
25		0.50		_
* CAG CCT ACC Gln Pro Thr	* * AAC CTC ACT CT Asn Leu Thr Le	rG CAT TAT TGC eu His Tyr Tr	TAC AAG AAC ' Tyr Lys Asn	TCG GAT AAT Ser Asp Asn>
290	300	310	320	330
* * GAT AAA GTC Asp Lys Val	CAG AAG TGC A	GC CAC TAT CT er His Tyr Le	A TTC TCT GAA u Phe Ser Glu	GAA ATC ACT Glu Ile Thr>
340	350	360	370	380
* * TCT GGC TGT Ser Gly Cys	CAG TTG CAA A	AAA AAG GAG AT Lys Lys Glu Il	C CAC CTC TAC e His Leu Tyr	CAA ACA TTT Gln Thr Phe>
390	400	410	420	430
* * GTT GTT CAG Val Val Glr	* * CTC CAG GAC (Leu Gln Asp	CCN CGG GAA C	CC AGG AGA CAG ro Arg Arg Gln	GCC ACA CAG Ala Thr Gln>
440	450	460	470 * . *	480 * *
* * ATG CTA AAA Met Leu Ly:	A CTG CAG AAT s Leu Gln Asn	CTG GTG ATC C Leu Val Ile F	CC TGG GCT CCA	A GAG AAC CTA o Glu Asn Leu>
	490	500 *	* *	520 * *
ACA CTT CA Thr Leu Hi	* * C AAA CTG AGT s Lys Leu Ser	GAA TCC CAG	CTA GAA CTG AA Leu Glu Leu As	C TGG AAC AAC n Trp Asn Asn
530	540	550	560	570 * *
AGA TTC TT	G AAC CAC TGT	TTG GAG CAC	TTG GTG CAG TA	C CGG ACT GAC

Figure 21B

580	590	600	*	610	620	
TGG GAC CAC Trp Asp His	AGC TGG ACT Ser Trp Thr	GAA CAA Glu Gln	TCA GTG Ser Val	GAT TAT Asp Tyr	AGA CAT AAG Arg His Lys	TTC Phe>
630	640	*	550	660	*	570 *
TCC TTG CCT Ser Leu Pro	AGT GTG GAT Ser Val Asp	GGG CAG	AAA CGC Lys Arg	TAC ACG Tyr Thr	TTT CGT GTT Phe Arg Val	r CGG l Arg>
680	690) * *	700 *	*	710	720 *
AGC CGC TTT Ser Arg Pho	AAC CCA CTO	TGT GGA Cys Gly	AGT GCT Ser Ala	CAG CAT Gln His	TGG AGT GAL	A TGG u Trp>
-	730	740	750		760	4
AGC CAC CC	* * A ATC CAC TG o Ile His Tr	* G GGG AGC p Gly Ser	AAT ACT	TCA AAA	. GAG AAC GC : Glu Asn Al	G TCG a Ser>
770	780	790		800	810	
* * TCT GGG AA Ser Gly As	.C ATG AAG GI n Met Lys Va	C CTG CAC	G GAG CCC	ACC TGG Thr Cys	GTC TCC GA Val Ser As	AC TAC sp Tyr>
820	830	840	0 *	850 *	* 860) *
ATG AGC AT Met Ser Il	CC TCT ACT TO Le Ser Thr C	GC GAG TGG	G AAG AT p Lys Me	G AAT GG t Asn Gl	T CCC ACC AM y Pro Thr A	AT TGC sn Cys>
870	880		890	90	0 * *	910
* * AGC ACC GA Ser Thr G	AG CTC CGC C lu Leu Arg L	TG TTG TA eu Leu Ty	C CAG CT	G GTT TT u Val Ph	T CTG CTC T ie Leu Leu S	CC GAA er Glu>
92	0 9	30	940	*	950	960
* GCC CAC A Ala His T	* CG TGT ATC Chr Cys Ile F	CT GAG AF	AC AAC GO an Aan G	GA GGC GC ly Gly A:	CG GGG TGC C la Gly Cys V	TG TGC Val Cys>
	970	980	9	90	1000	*
CAC CTG C His Leu I	TC ATG GAT (Leu Met Asp	GAC GTG G Asp Val V	TC AGT G al Ser A	CG GAT A la Asp A	AC TAT ACA o	CTG GAC Leu Asp>
1010	1020	1030	*	1040	1050	*
* CTG TGG (Leu Trp	GCT GGG CAG Ala Gly Gln	CAG CTG C	TG TGG A eu Trp I	AG GGC I	CC TTC AAG Ser Phe Lys	CCC AGC Pro Ser>
1060	1070	10	080	1090) 11 * *	L00 *
GAG CAT Glu His	GTG AAA CCC Val Lys Pro	AGG GCC C Arg Ala I	CCA GGA A	AAC CTG / Asn Leu '	ACA GTT CAC Thr Val His	ACC AAT Thr Asn>
1110	11.2	* *	1130 *	*	140	1150 *
כתר תרר	GAC ACT CTG Asp Thr Leu	CTG CTG	ACC TGG Thr Trp	AGC AAC Ser Asn	CCG TAT CCC Pro Tyr Pro	CCT GAC Pro Asp
1 :	160	1170	118	* *	1190	1200

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu> 1210 1220 1230 1240 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro> 1250 1260 1270 1280 1290 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg> 1300 1310 1320 1330 1340 GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC TGG AGT GAG. Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Trp Ser Glu> TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu> 1400 1410 1420 1430 * * * * * * * * * * CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 1450 1460 1470 1480 * * * * * * * * * CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> 1490 1500 1510 1520 * * * * * * * * * * ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp> 1540 1550 1560 1570 1580 * * * * * * * * * GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly> 1590 1600 1610 1620 1630 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn> 1640 1650 1660 1670 1680 * * * * * * * * * * * * AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp> 1690 1700 1710 1720 * * * * * * * CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> 1730 1740 1750 1760 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

Figure 21D

CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn> 1830 1840 1850 1860 1870 * * * * * * * * * * * CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr> 1930 1940 1950 1960 * * * * * * * * * * ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys> CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys> 2020 2030 2040 2050 2060 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu> 2070 2080 TCC CTG TCT CCG GGT AAA TGA Ser Leu Ser Pro Gly Lys ***>

Figure 22A

10	20	30	40 *	*
ATG GTG AAG CCA TCA Met Val Lys Pro Ser	TTA CCA TTC A	ACA TCC CTC Thr Ser Leu	TTA TTC CTG	CAG CTG Gln Leu>
50 60	70	80	90	*
CCC CTG CTG GGA GTG Pro Leu Leu Gly Val	GGG CTG AAC Gly Leu Asn	ACG ACA ATT	CTG ACG CCC Leu Thr Pro	AAT GGG Asn Gly>
100 110	120	*	30 1	40
AAT GAA GAC ACC ACA Asn Glu Asp Thr Thr	GCT GAT TTC Ala Asp Phe	TTC CTG ACC	ACT ATG CCC Thr Met Pro	ACT GAC Thr Asp>
	60 1	170	180	190
* * * * TCC CTC AGT GTT TCC Ser Leu Ser Val Ser	ACT CTG CCC	CTC CCA GAC	G GTT CAG TGT 1 Val Gln Cys	TTT GTG Phe Val>
200	210	220	230	240
* * * * TTC AAT GTC GAG TAG Phe Asn Val Glu Ty	ATG AAT TGC Met Asn Cys	ACT TGG AA	C AGC AGC TCT n Ser Ser Ser	GAG CCC Glu Pro>
250	260	270	280	*
CAG CCT ACC AAC CT Gln Pro Thr Asn Le	* C ACT CTG CAT u Thr Leu His	TAT TGG TA Tyr Trp Ty	C AAG AAC TCC r Lys Asn Sei	G GAT AAT c Asp Asn>
290 300	. 310	* 320	33(0 * *
GAT AAA GTC CAG AA Asp Lys Val Gln Ly	G TGC AGC CAC 's Cys Ser Hi:	C TAT CTA T s Tyr Leu Ph	C TCT GAA GA ne Ser Glu Gl	A ATC ACT u Ile Thr>
340 350	36	0 *	370	380 *
TCT GGC TGT CAG T Ser Gly Cys Gln L	rG CAA AAA AA eu Gln Lys Ly	G GAG ATC C s Glu Ile H	AC CTC TAC CA is Leu Tyr Gl	A ACA TTT n Thr Phe>
390	400	410	420	430
GTT GTT CAG CTC C Val Val Gln Leu G	AG GAC CCA CC ln Asp Pro Ar	GG GAA CCC A rg Glu Pro A	GG AGA CAG GG Arg Arg Gln A	CC ACA CAG la Thr Gln>
440	450 *	460 * *	470 * *	480
* * * ATG CTA AAA CTG C Met Leu Lys Leu C	AG AAT CTG G	TG ATC CCC 'al Ile Pro'	NGG GCT CCA G Frp Ala Pro G	AG AAC CTA lu Ásn Leu>
490	500 * *	510 * *	520 * *	*
ACA CTT CAC AAA (Thr Leu His Lys	CTG AGT GAA T Leu Ser Glu S	CC CAG CTA Ser Gln Leu	GAA CTG AAC 1 Glu Leu Asn 1	GG AAC AAC Trp Asn Asn>
530 540	550	5	60 *	570
AGA TTC TTG AAC Arg Phe Leu Asn	CAC TGT TTG (His Cys Leu (GAG CAC TTG Glu His Leu	GTG CAG TAC (Val Gln Tyr	CGG ACT GAC Arg Thr Asp>

580 590	600	610 *	620 * *
TGG GAC CAC AGC TGG A Trp Asp His Ser Trp T	CT GAA CAA TCA hr Glu Gln Ser	GTG GAT TAT Val Asp Tyr	AGA CAT AAG TTC Arg His Lys Phe>
630 640		660 * *	670
* * * TCC TTG CCT AGT GTG C	AAA OCO CAG AAA	CGC TAC ACG Arg Tyr Thr	TTT CGT GTT CGG Phe Arg Val Arg>
680	590	00	710 720
* * * AGC CGC TTT AAC CCA Ser Arg Phe Asn Pro	* CTC TGT GGA AGT Leu Cys Gly Se	GCT CAG CAT	TGG AGT GAA TGG Trp Ser Glu Trp>
730	740	750	760
AGC CAC CCA ATC CAC Ser His Pro Ile His	TGG GGG AGC AA Trp Gly Ser As	T ACT TCA AAA n Thr Ser Lys	GAG AAC GGG AAC Glu Asn Gly Asn>
770 780	790	800	* * * *
* * * ATG AAG GTC CTG CAG Met Lys Val Leu Gln	GAG CCC ACC TO	GC GTC TCC GAC	TAC ATG AGC ATC Tyr Met Ser Ile>
820 830	840	850 * *	860 * *
* * * TCT ACT TGC GAG TGG Ser Thr Cys Glu Trp	AND AND AND GO	GT CCC ACC AA ly Pro Thr As	T TGC AGC ACC GAG n Cys Ser Thr Glu>
870	80 89	0 90	0 910
* * * * CTC CGC CTG TTG TAG Leu Arg Leu Leu Ty:	C CAG CTG GTT T	TT CTG CTC TO	CC GAA GCC CAC ACG er Glu Ala His Thr>
920	930	940	950 960
* * *	* * * C AAC GGA GGC G n Asn Gly Gly	* * GCG GGG TGC G' Ala Gly Cys V	
970	980	990	1000
* * ATG GAT GAC GTG GT Met Asp Asp Val Va	* * CC AGT GCG GAT al Ser Ala Asp	* * AAC TAT ACA C Asn Tyr Thr I	TG GAC CTG TGG GCT Leu Asp Leu Trp Ala>
1010 1020	1030	1040	1050
* * * * GGG CAG CAG CTG C Gly Gln Gln Leu L	TG TGG AAG GGC eu Trp Lys Gly	TCC TTC AAG Ser Phe Lys	CCC AGC GAG CAT GTG Pro Ser Glu His Val>
1060 107		109	0 1100
* * AAA CCC AGG GCC C Lys Pro Arg Ala F		ACA GTT CAC Thr Val His	ACC AAT GTC TCC GAC Thr Asn Val Ser Asp>
1110	1120 1	130	.140 1150
* * * ACT CTG CTG CTG Thr Leu Leu Leu	* * ACC TGG AGC AAC Thr Trp Ser Asr	CCG TAT CCC	CCT GAC AAT TAC CTG Pro Asp Asn Tyr Leu>
1160	1170	1180	1190 1200

TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA AAC GAC CCG Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu Asn Asp Pro> GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC TCC CTC CGC Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro Ser Leu Arg> 50 1260 1270 1280 1290 ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG GCA CGG GTG Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg Ala Arg Val> AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG TGG AGC CCC Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu Trp Ser Pro> AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG CAG TCC GGA Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu Gln Ser Gly> 1400 1410 1420 1430 * * * * * * * * * GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly> 1450 1460 1470 1480 * * * * * * * GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met> 1490 1500 1510 1520 1530 * * * * * * * * * * * ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser His> 1540 1550 1560 1570 1580 * * * * * * * * * * * * GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val> 1590 1600 1610 1620 1630 * * * * * * * * * * * * CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr> 1640 1650 1660 1670 1680 * * * * * * * * * * * * CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly> 1690 1700 1710 1720 * * * * * * * * * * AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile> 1730 1740 1750 1760 1770 GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val>

TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser> CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu> 1880 1890 1900 1910 1920 * * * * * * * * * * * * TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro> 1930 1940 1950 1960 * * * * * * * * GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val> 1970 1980 1990 2000 2010 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met> 2020 2030 2040 2050 2060 CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser> 2070 CCG GGT AAA TGA Pro Gly Lys ***>

Figure 23A

	10	20	30	40	*
*	* * G CCA TCA TI	* የአርርኔ ጥጥር	ACA TCC CTC	TTA TTC C	TG CAG CTG
Met Val Ly	s Pro Ser Le	eu Pro Phe	Thr Ser Le	u Leu Phe L	eu Gln Leu>
50	60	70 * *	* *	*	90
CCC CTG CT Pro Leu Le	rG GGA GTG G eu Gly Val G	GG CTG AAC ly Leu Asn	ACG ACA AT Thr Thr Il	T CTG ACG (e Leu Thr I	CCC AAT GGG Pro Asn Gly>
100	110	120	•	130	140
* AAT GAA GA Asn Glu A	* AC ACC ACA G sp Thr Thr A	CT GAT TTC	TTC CTG AC	CC ACT ATG	CCC ACT GAC Pro Thr Asp>
150	160		170	180	190
* * TCC CTC A	* GT GTT TCC A er Val Ser T	* ACT CTG CCC Thr Leu Pro	C CTC CCA G	AG GTT CAG	
20		210	220	230	240
* TTC AAT C	* * GTC GAG TAC A	* * ATG AAT TG Met Asn Cv	* C ACT TGG A s Thr Trp A	AC AGC AGC	TCT GAG CCC Ser Glu Pro>
rile Asii V	250	260	270		80
CAG CCT A	* * ACC AAC CTC Thr Asn Leu	* ACT CTG CA Thr Leu Hi	T TAT TGG	AC AAG AAC Yr Lys Asn	TCG GAT AAT Ser Asp Asn>
290	300	310		20	330
* GAT AAA Asp Lys	* * * GTC CAG AAG Val Gln Lys	TGC AGC CA	AC TAT CTA ' is Tyr Leu	TTC TCT GAA Phe Ser Glu	GAA ATC ACT Glu Ile Thr>
340	350		60	370	380
TCT GGC Ser Gly	TGT CAG TTG Cys Gln Leu	CAA AAA A Gln Lys L	AG GAG ATC ys Glu Ile	CAC CTC TAC His Leu Ty:	C CAA ACA TTT r Gln Thr Phe>
390	. 4	00	410 *	420	430
* * * GTT GTT Val Val	CAG CTC CAG	GAC CCA C	GG GAA CCC	AGG AGA CA Arg Arg Gl	G GCC ACA CAG n Ala Thr Gln>
	440	450	460	470	480
ATG CTA Met Lev	AAA CTG CAG Lys Leu Gl	G AAT CTG (GTG ATC CCC Val Ile Pro	TGG GCT CC	CA GAG AAC CTA co Glu Asn Leu>
	490	500	510 * *	*	520
ACA CT Thr Le	r CAC AAA CT u His Lys Le	G AGT GAA u Ser Glu	TCC CAG CTA Ser Gln Lev	GAA CTG A Glu Leu A	AC TGG AAC AAC sn Trp Asn Asn>
530	540	55	* *	560	570 * *
AGA TT Arg Ph	C TTG AAC CA	C TGT TTG	GAG CAC TTO	G GTG CAG T u Val Gln T	'AC CGG ACT GAC 'yr Arg Thr Asp

32/74 Figure 23B

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580 590 600 610 620
* * * * * * * * * *
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>
630 640 650 660 670
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>
     680 690 700 710 720
* * * * * * * * * * *
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>
    730 740 750 760
* * * * * * * * * *
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
 Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>
 TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
 Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>
 820 830 840 850 860
 ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
 Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>
 870 880 890 900 910
* * * * * * * * * * *
 AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
  Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>
  920 930 940 950 960
* * * * * * * * * * *
  GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
  Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>
      970 980 990 1000
* * * * * * * * * *
  CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
  His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>
 1010 1020 1030 1040 1050
   CTG TGG GCT GGG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
   Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>
   1060 1070 1080 1090 1100
   GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
   Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>
   1110 1120 1130 1140 1150
* * * * * * * * * *
   GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
   Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>
       1160 1170 1180 1190
```

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu> 1220 1230 1240 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro> 1250 1260 1270 1280 1290 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg> 1300 1310 1320 1330 1340 GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu> 1350 1360 1370 1380 1390 * * * * * * * * * * * * TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu> 1400 1410 1420 1430 1440 * * * * * * * * * * * CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 1450 1460 1470 1480 * * * * * * * CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp> 1540 1550 1560 1570 1580 * * * * * * * * * * * * GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly> 1590 1600 1610 1620 1630 * * * * * * * * * * * * GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn> 1640 1650 1660 1670 1680 * * * * * * * * * * * * AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp> 1690 1700 1710 1720 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

1780 1790 1800 1810 1820 * * * * * * * * * * CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn> 1830 1840 1850 1860 1870 * * * * * * * * * * * CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> 1880 1890 1900 1910 1920 * * * * * * * * * * * * GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr> 1930 1940 1950 1960 * * * * * * * * * * ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys> 1970 1980 1990 2000 2010 CTC ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAC GTC TTC TCA TGC Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys> 2020 2030 2040 2050 2060 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu> 2070 2080 TCC CTG TCT CCG GGT AAA TGA Ser Leu Ser Pro Gly Lys ***>

10	20	30	40 *	*
* * * * ATG GTG GCC GTC Met Val Ala Val	GGC TGC GCG CTG Gly Cys Ala Leu	CTG GCT GCC Leu Ala Ala	CTG CTG GCC C Leu Leu Ala A	GCG CCG Ala Pro>
50 60	70	* *	90 * *	*
GGA GCG GCG CTG Gly Ala Ala Leu	GCC CCA AGG CGC Ala Pro Arg Arg	TGC CCT GCG Cys Pro Ala	CAG GAG GTG Gln Glu Val	GCA AGA Ala Arg>
100	110 120	*	* *	.40
GGC GTG CTG ACC	AGT CTG CCA GGA Ser Leu Pro Gly	GAC AGC GTG Asp Ser Val	ACT CTG ACC Thr Leu Thr	TGC CCG Cys Pro>
150	160	170	180	190
GGG GTA GAG CCC Gly Val Glu Pro	G GAA GAC AAT GCC	C ACT GTT CAC a Thr Val His	TGG GTG CTC Trp Val Leu	AGG AAG Arg Lys>
200	210	220	230 * *	240
CCG GCT GCA GG Pro Ala Ala Gl	C TCC CAC CCC AG Y Ser His Pro Se	C AGA TGG GC r Arg Trp Al	T GGC ATG GGA a Gly Met Gly	AGG AGG Arg Arg>
250	260	270 * *	280 * *	*
CTG CTG CTG AC	* * GG TCG GTG CAG CT rg Ser Val Gln Le	TC CAC GAC TO Bu His Asp Se	CT GGA AAC TA' er Gly Asn Ty	T TCA TGC r Ser Cys>
230	310	•	* *	* *
TAC CGG GCC G Tyr Arg Ala G	* * * * GC CGC CCA GCT G ly Arg Pro Ala G	GG ACT GTG C ly Thr Val H	AC TTG CTG G1 is Leu Leu Va	Al Asp Val>
340		360 * *	370	380 *
CCC CCC GAG C Pro Pro Glu C	* * SAG CCC CAG CTC Slu Pro Gln Leu S	TCC TGC TTC (Ser Cys Phe)	CGG AAG AGC C Arg Lys Ser P	CC CTC AGC ro Leu Ser>
390	400	410	420 * *	430
* * AAT GTT GTT ' Asn Val Val	TGT GAG TGG GGT Cys Glu Trp Gly	CCT CGG AGC Pro Arg Ser	ACC CCA TCC C Thr Pro Ser I	TG ACG ACA Leu Thr Thr>
440	450	460 * *	470 * *	480
AAG GCT GTG Lys Ala Val	CTC TTG GTG AGG Leu Leu Val Arg	AAG TTT CAG Lys Phe Gln	AAC AGT CCG (Asn Ser Pro	GCC GAA GAC Ala Glu Asp>
	500	510 * *	52 *	* *
TTC CAG GAG Phe Gln Glu	CCG TGC CAG TAT Pro Cys Gln Tyr	TCC CAG GAG Ser Gln Glu	TCC CAG AAG Ser Gln Lys	TTC TCC TGC Phe Ser Cys>
530	540	550 * *	560	570 * *
CAG TTA GCA Gln Leu Ala	GTC CCG GAG GGA Val Pro Glu Gly	A GAC AGC TCT y Asp Ser Ser	TTC TAC ATA Phe Tyr Ile	GTG TCC ATG Val Ser Met>

Figure 24B

580	590		600		610	620	5
TGC GTC GC Cys Val Al	* * CC AGT AGT La Ser Ser	GTC GGG Val Gly	AGC AAC Ser Lys	* G TTC A G Phe S	GC AAA A er Lys T	CT CAA AC	CC TTT hr Phe>
630	• 6	40	650 *	*	660	*	670 *
CAG GGT TO	GT GGA ATO	TTG CAG	CCT GA'	r CCG C p Pro P	CT GCC /	AC ATC A Asn Ile T	CA GTC hr Val>
68	0 *	690	*	700 *	* 7:	10	720
ACT GCC G	TG GCC AGA al Ala Arg	A AAC CCC g Asn Pro	CGC TG Arg Tr	G CTC / p Leu S	AGT GTC . Ser Val '	ACC TGG C Thr Trp G	AA GAC In Asp>
	730	740	*	750 *	*	760 *	*
CCC CAC T Pro His S	CC TGG AAGET Trp As	TCA TC	T TTC TA	C AGA (CTA CGG Leu Arg	TTT GAG (Phe Glu I	TC AGA Leu Arg>
770	780		790 *	8	00	810	*
TAT CGG C	CT GAA CG	G TCA AA g Ser Ly	G ACA TT	TC ACA	ACA TGG Thr Trp	ATG GTC A	AAG GAC Lys Asp>
820	830		840	•	850 *	. 8	60
CTC CAG (Leu Gln)	* * CAT CAC TO His His C	T GTC AT	C CAC G	AC GCC sp Ala	TGG AGC	GGC CTG	AGG CAC Arg His>
870	_	880	89	0	900	*	910
GTG GTG Val Val	CAG CTT CO Gln Leu A	GT GCC CA	AG GAG G Ln Glu G	AG TTC	GGG CAA Gly Gln	GGC GAG Gly Glu	TGG AGC Trp Ser>
9	20	930		940		950	960
GAG TGG Glu Trp	* AGC CCG G Ser Pro G	AG GCC A	rG GGC # et Gly 1	CG CCT	TGG ACA	GAA TCC Glu Ser	AGG AGT
	970	98	0	990		1000	•
CCT CCA Pro Pro	GCT GAG A	.AC GAG G .sn Glu V	TG TCC A	ACC CCC Thr Pro	ATG ACC	GGT GGC Gly Gly	GCG CCT Ala Pro>
1010	1020		1030	. 1	1040	1050	*
* TCA GGT Ser Gly	GCT CAG (Ala Gln)	CTG GAA (Leu Glu I	CTT CTA Leu Leu	GAC CCA Asp Pro	A TGT GG Cys Gl	TAT ATC	
1060	10	70	1080	*	1090	*	1100
GAA TCT	CCA GTT	GTA CAA (Val Gln	CTT CAT Leu His	TCT AA	T TTC AC	T GCA GT	r TGT GTG l Cys Val>
1110) * *	1120	. 13	L30 *	114	10	1150
CTA AAG	G GAA AAA	TGT ATG	GAT TAT Asp Tyr	TTT CA	T GTA AA .s Val Aa	AT GCT AA sn Ala As	T TAC ATT n Tyr Ile>
•	1160	1170	*	1180	*	1190	1200

Figure 24C

GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT ACT ATC Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile> 1210 1220 1230 1240 ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT TCA TTA Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu> 1260 1270 1280 1290 AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT GAA CAG Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln> AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro> AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu> 1400 1410 1420 1430 TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys> 1450 1460 1470 1480 TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA CGT GAC Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp> 1490 1500 1510 1520 ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT GTC AAC Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn> 1540 1550 1560 1570 1580 * * * * * * * * * * * * ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT ACA TCA Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser> 1590 1600 1610 1620 1630 GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT CCG CCA Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro> 1640 1650 1660 1670 * * * * * * * * * * CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys> TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr> 1740 1750 1760 * * * * * * * * AAC ATT CAA TAT AGG ACC AAA. GAT GCC TCA ACT TGG AGC CAG ATT CCT Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro>

Figure 24D

1780	1790	1800	1810	182	0
* CCT GAA GAG Pro Glu Ası	* C ACA GCA TCC C Thr Ala Se	* * * C ACC CGA TO Thr Arg So	* * CT TCA TTC AC er Ser Phe Tl	* CT GTC CAA G nr Val Gln A	* AC CTT sp Leu>
1830	1840	185	0 1.8	60	1870
AAA CCT TT Lys Pro Ph	T ACA GAA TA e Thr Glu Ty	T GTG TTT A r Val Phe A	GG ATT CGC T rg Ile Arg C	GT ATG AAG G ys Met Lys G	SAA GAT Slu Asp>
1880	189 *	0 * *	1900	1910	1920
GGT AAG GG Gly Lys Gl	A TAC TGG AG y Tyr Trp Se	T GAC TGG A	GT GAA GAA G Ser Glu Glu A	CA AGT GGG A	ATC ACC Ile Thr>
	.930	1940	1950	1960	*
TAT GAA GA Tyr Glu As	AT AGA CCA TO sp Arg Pro So	CT AAA GCA (er Lys Ala 1	CCA AGT TTC T Pro Ser Phe S	TGG TAT AAA . Trp Tyr Lys	ATA GAT Ile Asp>
1970	1980 *	1990 * *	2000 * *	2010	*
CCA TCC C Pro Ser H	AT ACT CAA G is Thr Gln G	GC TAC AGA ly Tyr Arg	ACT GTA CAA Thr Val Gln	CTC GTG TGG Leu Val Trp	AAG ACA Lys Thr>
2020	2030	2040	205 *	0 20)60 *
TTG CCT C Leu Pro P	CT TTT GAA C ro Phe Glu A	CC AAT GGA la Asn Gly	AAA ATC TTG Lys Ile Leu	GAT TAT GAA Asp Tyr Glu	GTG ACT Val Thr>
2070	2080	20	90 * *	100	2110
CTC ACA A Leu Thr A	AGA TGG AAA : Arg Trp Lys :	CCA CAT TTA Ser His Leu	CAA AAT TAC Gln Asn Tyr	ACA GTT AAT Thr Val Asn	GCC ACA Ala Thr>
212	20 2	130	2140	2150	2160 * *
AAA CTG A	ACA GTA AAT Thr Val Asn	CTC ACA AAT Leu Thr Asn	GAT CGC TAT Asp Arg Tyr	CTA GCA ACC Leu Ala Thr	CTA ACA
*	2170	2180	2190	2200 * *	*
GTA AGA Val Arg	AAT CTT GTT Asn Leu Val	GGC AAA TCA Gly Lys Ser	GAT GCA GCT Asp Ala Ala	GTT TTA ACT Val Leu Thi	r ATC CCT r Ile Pro>
2210	2220	2230	2240	225°	* , *
GCC TGT Ala Cys	GAC TTT CAA Asp Phe Gln	GCT ACT CAC Ala Thr His	CCT GTA ATO Pro Val Met	GAT CTT AA Asp Leu Ly	A GCA TTC s Ala Phe>
2260	2270 * *	228	* *	290 * *	2300
CCC AAA Pro Lys	GAT AAC ATG Asp Asn Met	CTT TGG GT Leu Trp Va	G GAA TGG AC' l Glu Trp Th	T ACT CCA AC r Thr Pro Ar	GG GAA TCT g Glu Ser>
2310	*	20	2330	2340	2350
GTA AAG Val Lys	AAA TAT ATA Lys Tyr Ile	CTT GAG TG	G TGT GTG TT p Cys Val Le	A TCA GAT AA au Ser Asp Ly	AA GCA CCC ys Ala Pro>
2	360	2370	2380	2390	2400

Figure 24E

TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr> 2410 2420 2430 2440 TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr> 2460 2470 2480 2490 CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG GCA TAC Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala Tyr> 2510 2520 2530 2540 CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA AAA AAA Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys Lys> GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT GTT GAT Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val Asp> 2600 2610 2620 2630 2640 GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA ACC ATC Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr Ile> 2650 2660 2670 * * * * * * * ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu Tyr> 2690 2700 2710 2720 2730 ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala> 2750 2760 2770 * * * * * * GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr> 2790 2800 2810 2820 2830 * * * * * * * * * * * * * * ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA TCC GGG GGC GAC AAA ACT Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ser Gly Gly Asp Lys Thr> 2850 2860 2870 2880 * * * * * * * * * CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser> 2890 2900 2910 GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg> 2950 2960 2970 2940 2950 ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT

WO 00/18932 Figure 24F

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Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>
            2990 3000 3010 3020
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>
   3030 3040 3050 3060 3070
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>
      3080 3090 3100 3110 3120
 AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>
        3130 3140 3150
 AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>
3170 3180 3190 3200 3210
 ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>
       3230 3240 3250
  CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC
  Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys>
  3270 3280 3290 3300 3310
* * * * * * * * * * * *
  CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
  Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>
       3320 3330 3340 3350 3360
  AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC
  Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>
          3370 3380 3390 3400
   TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC
   Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>
            3420 3430 3440 3450
 3410
   AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
   Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>
    3460 3470 3480 3490 3500
   CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
   Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>
    TGA
```

Figure 25A

10	20	*	30	40	*
ATG GTG GCC GT Met Val Ala Va	C GGC TGC GC l Gly Cys Al	G CTG CTG a Leu Leu	GCT GCC CTC Ala Ala Le	G CTG GCC G u Leu Ala A	CG CCG
50 6	0	70	80	90 * *	*
GGA GCG GCG CT Gly Ala Ala Le	CG GCC CCA AC au Ala Pro Ai	GG CGC TGC	CCT GCG CA Pro Ala Gl	G GAG GTG (n Glu Val A	GCA AGA Ala Arg>
100	110	120	130	1.	40 *
GGC GTG CTG AGGLY Val Leu T	CC AGT CTG Chr Ser Leu P	CA GGA GAC ro Gly Asp	AGC GTG AC	T CTG ACC	TGC CCG Cys Pro>
150	160	170	1	80	190 *
* * GGG GTA GAG C	* CG GAA GAC A ro Glu Asp A	AT GCC ACT	GTT CAC TO	GG GTG CTC rp Val Leu	AGG AAG Arg Lys>
200	210		220	230	240
CCG GCT GCA C	* * GC TCC CAC G Gly Ser His	CCC AGC AG	A TGG GCT G g Trp Ala G	GC ATG GGA Sly Met Gly	AGG AGG Arg Arg>
250	2	60	270	280	*
* CTG CTG CTG Leu Leu Leu	* * AGG TCG GTG Arg Ser Val	* CAG CTC CA Gln Leu Hi	C GAC TCT (s Asp Ser (GGA AAC TAT Gly Asn Tyr	TCA TGC Ser Cys>
290	300	310	320	* *) : *
* * TAC CGG GCC Tyr Arg Ala	GGC CGC CCA Gly Arg Pro	GCT GGG AC	T GTG CAC	TTG CTG GTG Leu Leu Val	GAT GTT L Asp Val>
340	350	360	*	'0 * *	380
CCC CCC GAG Pro Pro Glu	GAG CCC CAG Glu Pro Gln	CTC TCC To	GC TTC CGG ys Phe Arg	AAG AGC CC Lys Ser Pr	C CTC AGC o Leu Ser>
390	400	41	0 *	420	430
* * AAT GTT GTT Asn Val Val	TGT GAG TGG Cys Glu Trp	GGT CCT C	GG AGC ACC Arg Ser Thr	CCA TCC CI Pro Ser Le	G ACG ACA au Thr Thr>
440	450) . *	460	470 *	. 480
* * AAG GCT GTC Lys Ala Val	CTC TTG GTG	G AGG AAG ' L Arg Lys '	TTT CAG AAC Phe Gln Asn	AGT CCG GG Ser Pro A	CC GAA GAC la Glu Asp>
	490	500	510	520 * *	*
* TTC CAG GAO Phe Gln Gl	* * G CCG TGC CA u Pro Cys Gl	G TAT TCC n Tyr Ser	CAG GAG TCG Gln Glu Se	CAG AAG T	TC TCC TGC he Ser Cys
530	540	550	560	\$ *	570 * *
CAG TTA GC	A GTC CCG GA a Val Pro Gl	G GGA GAC	AGC TCT TT Ser Ser Ph	C TAC ATA C e Tyr Ile \	GTG TCC ATG Val Ser Met

Figure 25B

580	590	600	610	620
* * TGC GTC GCC Cvs Val Ala	* AGT AGT GTC Ser Ser Val	* * GGG AGC AAG Gly Ser Lys	TTC AGC AAA Phe Ser Lys	ACT CAA ACC TTT Thr Gln Thr Phe>
630	640	650	660	670
* *	* * GGA ATC TTC Gly Ile Lev	* * * G CAG CCT GAT G Gln Pro Asj	* * * CCG CCT GCC Pro Pro Ala	* *AAC ATC ACA GTC Asn Ile Thr Val>
680	690		700	710 720
* * ACT GCC GTG Thr Ala Va	* G GCC AGA AA l Ala Arg As	*	G CTC AGT GTC p Leu Ser Va	C ACC TGG CAA GAC l Thr Trp Gln Asp>
	730	740	750	760
* CCC CAC TC Pro His Se	* * C TGG AAC TC	* * A TCT TTC TA r Ser Phe Ty	.C AGA CTA CG vr Arg Leu Ar	G TTT GAG CTC AGA g Phe Glu Leu Arg>
770	780	790	800	810
* * TAT CGG GO Tyr Arg A	* CT GAA CGG TC La Glu Arg Sc	* CA AAG ACA T' er Lys Thr P	TC ACA ACA TO he Thr Thr Ti	GG ATG GTC AAG GAC p Met Val Lys Asp>
820	830	840	850 * *	860 * *
* CTC CAG C Leu Gln H	* AT CAC TGT G is His Cys V	שם אשר כאר פ "	AC GCC TGG A	GC GGC CTG AGG CAC er Gly Leu Arg His>
870	880		_	00 910
* * GTG GTG C Val Val C	* AG CTT CGT (In Leu Arg A	SCC CAG GAG (Ala Gln Glu (GAG TTC GGG C	AA GGC GAG TGG AGC In Gly Glu Trp Ser>
		930	940	950 960 * * *
* GAG TGG A	* * AGC CCG GAG Ser Pro Glu	* * GCC ATG GGC Ala Met Gly	ACG CCT TGG A	ACA GAA TCG CGA TCG Thr Glu Ser Arg Ser>
•	970	980	990	1000
* CCT CCA Pro Pro	* * GCT GAG AAC Ala Glu Asn	* GAG GTG TCC Glu Val Ser	ACC CCC ATG Thr Pro Met	GAA CTT CTA GAC CCA Glu Leu Leu Asp Pro>
1010	1020	1030	1040	1050
* TGT GGT Cys Gly	* * TAT ATC AGT Tyr Ile Ser	CCT GAA TCT Pro Glu Ser	CCA GTT GTA Pro Val Val	CAA CTT CAT TCT AAT Gln Leu His Ser Asn>
1060	1070	1080	10	90 1100
* TTC ACT Phe Thr	GCA GTT TGT	GTG CTA AAG Val Leu Lys	GAA AAA TGT Glu Lys Cys	ATG GAT TAT TTT CAT Met Asp Tyr Phe His:
1110	, 11	.20	1130	1140 1150
* * * GTA AAT Val Ası	* C GCT AAT TAG Ala Asn Ty:	ATT GTC TGC	G AAA ACA AAC p Lys Thr Asr	C CAT TTT ACT ATT CCT h His Phe Thr Ile Pro
	1160	1170	1180	1190 1200

Figure 25C

```
AAG GAG CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT
 Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe>
        1210 1220 1230 1240
 ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA
 Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr>
1250 1260 1270 1280
 TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC
 Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly>
       1310 1320 1330 1340
  TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG
  Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly>
    1350 1360 1370 1380
  AAG AAA ATG AGG TGT GAG TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG
  Lys Lys Met Arg Cys Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu>
       ACA AAC TTC ACT TTA AAA TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT
  Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp>
          1450 1460 1470 1480
  TGC AAA GCA AAA CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT
  Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser>
 ACT GTG TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC
   Thr Val Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala>
     540 1550 1560 1570 1580
* * * * * * * * * * *
   CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA
   Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys>
   GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA
   Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu>
              1650 1660 1670 1680
* * * * * * * * * *
    CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT
    Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser>
           1690 1700 1710 1720
* * * * * * * *
    GTT ATA ATA CTA AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA
    Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser>
             1740 1750 1760 1770 * * * * * *
    ACT TGG AGC CAG ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA
    Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser>
```

		1000	1810	1820	
1780 *	1790 * *	1800	* *	* *	ው ጥ
TTC ACT G Phe Thr V	TC CAA GAC CT al Gln Asp Le	r AAA CCT TT 1 Lys Pro Phe	Thr Glu Ty	r Val Phe Arg	Ile>
1830	1840	1850	186	0 18 * *	170 *
* * CGC TGT A Arg Cys N	ATG AAG GAA GA Met Lys Glu As	T GGT AAG GG. p Gly Lys Gl	N TNC TGG NG y Tyr Trp Se	T GAC TGG AGT r Asp Trp Sei	GAA Glu>
188	30 189	0 1	900	1910	1920 *
GAA GCA A	* AGT GGG ATC AC Ser Gly Ile Ti	C TAT GAA GA ar Tyr Glu As	T AGA CCA TO p Arg Pro Se	T AAA GCA CC T Lys Ala Pro	A AGT o Ser>
	1930	1940	1950	1960 * *	*
* TTC TGG Phe Trp	TAT AAA ATA G Tyr Lys Ile A	את ככא שככ כז	AT ACT CAA G is Thr Gln G	GC TAC AGA AC ly Tyr Arg Th	T GTA r Val>
1970	1980	1990	2000	2010	*
* CAA CTC Gln Leu	* * GTG TGG AAG A Val Trp Lys T	CA TTG CCT C hr Leu Pro P	CT TTT GAA G	CC AAT GGA AA la Asn Gly Ly	AA ATC ys Ile>
2020	2030	2040	2050		0 *
* TTG GAT Leu Asp	TAT GAA GTG A	ACT CTC ACA A	GA TGG AAA T Arg Trp Lys S	CCA CAT TTA C Ser His Leu G	AA AAT ln Asn>
2070	208	209	20 *	100	2110
* * TAC ACA Tyr Thr	GTT AAT GCC Val Asn Ala	ACA AAA CTG Thr Lys Leu	ACA GTA AAT Thr Val Asn	CTC ACA AAT G Leu Thr Asn A	ASP Arg>
		130	2140	2150	2160
* TAT CT Tyr Le	* * A GCA ACC CTA u Ala Thr Leu	ACA GTA AGA Thr Val Arg	AAT CTT GTT Asn Leu Val	GGC AAA TCA G Gly Lys Ser	GAT GCA Asp Ala>
	2170	2180	2190	2200	*
* GCT GT Ala Va	* * T TTA ACT ATC 1 Leu Thr Ile	CCT GCC TGT Pro Ala Cys	GAC TTT CAA Asp Phe Gln	GCT ACT CAC	CCT GTA Pro Val>
2210	2220	2230	2240	2250	*
* ATG GA Met As	* * AT CTT AAA GCA sp Leu Lys Ala	mmc ccc AAA	GAT AAC ATG Asp Asn Met	CTT TGG GTG Leu Trp Val	GAA TGG Glu Trp>
2260	2270	2280		290 * *	300 *
* ACT A Thr T	T CCA AGG GAI hr Pro Arg Gl	. መርመ ርመን አእር	ער היא האים ממים בי	A CTT GAG TGG e Leu Glu Trp	TGT GTG Cys Val>
23	10 2	320	2330	2340	2350
* TTA T Leu S	* * CCA GAT AAA GC Ser Asp Lys Al	አ ድድድ ጥርጥ አ ጥ	C ACA GAC TG e Thr Asp Tr	G CAA CAA GAA p Gln Gln Gl	A GAT GGT 1 Asp Gly>
	2360	2370	2380	2390	2400

Figure 25E

ACC GTG CAT CGC ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC Thr Val His Arg Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys> 2420 2430 2440 TAT TTG ATA ACA GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT Tyr Leu Ile Thr Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro> 2460 2470 2480 2490 * * * * * * * * GAA TCC ATA AAG GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT Glu Ser Ile Lys Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro> 2510 2520 2530 ACT GTT CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG Thr Val Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp> 2550 2560 2570 2580 2590 * * * * * * * * * * * * GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr> 2600 2610 2620 2630 2640 * * * * * * * * * * * ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp> 2650 2660 2670 2680 TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG Ser Ser His Thr Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu> 2690 2700 2710 2720 TAC ATG GTA CGA ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT Tyr Met Val Arg Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly> 2740 2750 2760 * * * * * * * CCA GAA TTC ACT TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA Pro Glu Phe Thr Phe Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu> 2790 2800 2810 2820 2830 * * * * * * * * * * * * * TCC GGG GGC GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Ser Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 2850 2860 2870 . 2880 2850 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> 2890 2900 2910 2920 ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp> 2950 2960 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC

Figure 25F

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly> 2990 3000 3010 3020 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn> 3030 3040 3050 3060 3070 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp> 3080 3090 3100 3110 * CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> 3130 3140 3150 3160 * * * * * * * * * * GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu> 3170 3180 3190 3200 3210 * * * * * * * * * * * CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn> 3220 3230 3240 3250 3260 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> 3270 3280 3290 3300 3310 GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr> 3320 3330 3340 3350 * * * * * * * * * * ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys> 3370 3380 3390 3400 * * * * * * * * * CTC ACC GTG GAC AAG AGC AGG TGG CAG GGG AAC GTC TTC TCA TGC Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys> 3410 3420 3430 3440 3450 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu> 3460 3470 TCC CTG TCT CCG GGT AAA TGA Ser Leu Ser Pro Gly Lys ***>

Figure 26A

10	•	20	30 *	40 * *	*
ATG GTG CTT C	TG TGG TGT eu Trp Cys	GTA GTG AG	r CTC TAC ' r Leu Tyr !	TTT TAT GGA Phe Tyr Gly	ATC CTG Ile Leu>
50	60	70	* *	90 * *	*
CAA AGT GAT G	CC TCA GAA la Ser Glu	CGC TGC GA	T GAC TGG p Asp Trp	GGA CTA GAC Gly Leu Asp	ACC ATG Thr Met>
100	110	120	13	0 * *	140
AGG CAA ATC C	CAA GTG TTT Gln Val Phe	GAA GAT GA Glu Asp Gl	G CCA GCT u Pro Ala	CGC ATC AAG Arg Ile Lys	TGC CCA Cys Pro>
150	160	170		180	190 *
* * CTC TTT GAA Leu Phe Glu	CAC TTC TTC His Phe Lev	AAA TTC A	AC TAC AGC on Tyr Ser	ACA GCC CAT Thr Ala His	T TCA GCT S Ser Ala>
200	210	0	220	230	240
GGC CTT ACT Gly Leu Thr	CTG ATC TG	G TAT TGG A p Tyr Trp T	CT AGG CAG hr Arg Gln	GAC CGG GAG Asp Arg As	C CTT GAG p Leu Glu>
25	50	260	270	280 * *	*
GAG CCA ATT Glu Pro Ile	AAC TTC CG Asn Phe Ar	C CTC CCC C	AG AAC CGC	ATT AGT AA Ile Ser Ly	G GAG AAA s Glu Lys>
290	300	310	320	33	30 * *
GAT GTG CTG Asp Val Leu	TGG TTC CC	GG CCC ACT (CTC CTC AAT Leu Leu Asi	r GAC ACT GO n Asp Thr G	GC AAC TAT Ly Asn Tyr>
340	350	360	*	370	380 *
ACC TGC ATG Thr Cys Met	TTA AGG A Leu Arg A	AC ACT ACA sn Thr Thr	TAT TGC AG Tyr Cys Se	C AAA GTT G r Lys Val A	CA TTT CCC la Phe Pro>
390	400	*	10	420 *	430
TTG GAA GTT Leu Glu Val	GTT CAA A	AA GAC AGC ys Asp Ser	TGT TTC AA Cys Phe As	AT TCC CCC Asn Ser Pro M	TG AAA CTC let Lys Leu>
440	4	150	460	470 * *	. 480 * *
CCA GTG CA' Pro Val Hi	T AAA CTG T s Lys Leu T	MAT ATA GAA Nyr Ile Glu	TAT GGC AT Tyr Gly I	TT CAG AGG A le Gln Arg	ATC ACT TGT Ile Thr Cys>
_	490	500	510	5 2	0 * *
CCA AAT GT Pro Asn Va	A GAT GGA	TAT TTT CCT Tyr Phe Pro	TCC AGT G Ser Ser V	TC AAA CCG al Lys Pro	ACT ATC ACT Thr Ile Thr>
530	540	550 * *	56 *	* *	570
TGG TAT AT	rG GGC TGT	TAT AAA ATA Tyr Lys Ile	CAG AAT T	TTT AAT AAT The Asn Asn	GTA ATA CCC Val Ile Pro>

48/*74* Figure 26B

580 590 600 610 620 * * * * * * * * * * GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT GGA Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly> 630 640 650 660 670 AAT TAC ACA TGT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT CAT Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His> 680 690 700 710 720 * * * * * * * * * * * CTC ACC AGG ACT CTG ACT GTA AAG GTA GTA GGC TCT CCA AAA AAT GCA Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala> 730 740 750 760 * * * * * * * * * * GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG AAA Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys> GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT TTT Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe> 820 830 840 850 860 * * * * * * * CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA AAA Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys> 870 880 890 900 910 * * * * * * * * * * * CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT CAT Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His> 920 930 940 950 960 * * * * * * * * * * * AGT AGA ACA GAA GAT GAA ACA AGA ACT CAG ATT TTG AGC ATC AAG AAA Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys> 970 980 990 1000 * * * * * * * * * * GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA AGT Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser> 1010 1020 1030 1040 1050 GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GTG CCA Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro> 1060 1070 1080 1090 1100 GCT CCA AGA TAC ACA GTG TCC GGT GGC GCG CCT ATG CTG AGC GAG GCT Ala Pro Arg Tyr Thr Val Ser Gly Gly Ala Pro Met Leu Ser Glu Ala> 1110 1120 1130 1140 1150 GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA ATT TTA GTG TCA TCT GCA Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val Ser Ser Ala> 1160 1170 1180 1190 1200

Figure 26C

AAT GAA ATT GAT GTT CGT CCC TGT CCT CTT AAC CCA AAT GAA CAC AAA Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn Glu His Lys> 1210 1220 1230 1240 GGC ACT ATA ACT TGG TAT AAG GAT GAC AGC AAG ACA CCT GTA TCT ACA Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro Val Ser Thr> 1250 1260 1270 1280 1290 GAA CAA GCC TCC AGG ATT CAT CAA CAC AAA GAG AAA CTT TGG TTT GTT Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu Trp Phe Val> 1300 1310 1320 1330 1340 CCT GCT AAG GTG GAG GAT TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val Val Arg Asn> 1350 1360 1370 1380 1390 * * * * * * * * * * * * TCA TCT TAC TGC CTC AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn> 1400 1410 1420 1430 1440 GAG CCT AAC TTA TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu> 1450 1460 1470 1480 CCC GTT GCA GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr Met Glu Phe Phe> 90 1500 1510 1520 1530 AAA AAT GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys> 1550 1560 1570 1580 * * * * * * * * * AAA CCT CTA CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg> 1590 1600 1610 1620 CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT TGT Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr Cys> 1640 1650 1660 1670 * * * * * * * * * CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC CGG GTA His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr Arg Val> 1690 1700 1710 1720 * * * * * * * * ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG CCT GTG ATT Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg Pro Val Ile> 30 1740 1750 1760 1770 * * * * * * * * * * * * GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG GGA TCC CAG ATA

Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly Ser Gln Ile>

Figure 26D

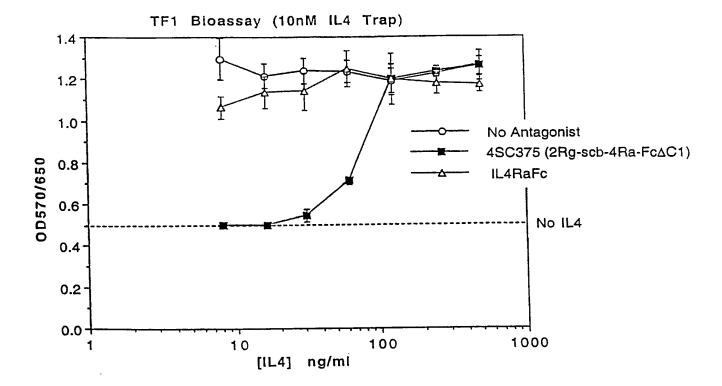
1780	.	17	90		. 1	800		*	181	0	*	18	20		
CAA TTG Gln Leu	ATC T	rgr Cys	AAT Asn	GTC Val	ACC Thr	GGC Gly	CAG Gln	TTG Leu	AGT Ser	GAC Asp	Ile	GCT Ala	TAC Tyr	TGG Trp>	
1830			184	0		18	350		*	860		*	187	70 *	
* * AAG TGG Lys Trp	AAT (* GGG Gly	TCA Ser	GTA Val	ATT Ile	GAT Asp	GAA Glu	GAT Asp	GAC	CCA Pro	GTG Val	СТЛ	GGG Gly	GAA Glu>	•
1.8	380			1890			19	00		1	910			1920	
* GAC TAT Asp Tyr	* TAC Tyr	AGT Ser	* GTG Val	GAA Glu	TAA Asn	CCT Pro	GCA Ala	AAC Asn	AAA Lys	AGA Arg	AGG Arg	AGT Ser	ACC Thr	CTC Leu:	>
	193	0		1	940		*	1950	.	*	19	60	*		
ATC ACA Ile Thr	GTG Val	* CTT Leu	AAT Asn	ATA Ile	. TCG Ser	GAA Glu	ATT	GAG	AGT Ser	AGA Arg	TTI Phe	TAT TYP	AAA Lys	CAT His	>
1970	. 1	1980			19	90	,	. 2	2000		*	2010)	*	
* CCA TTT Pro Phe	* ACC Thr	TGT Cys	TTT	GCC Ala	AAC a Ly:	AA:	r ACA	A CAT	r GG s Gly	r ATZ Y Ile	A GA' e Asi	r GCA p Ala	A GCA A Ala	A TAI	; ;>
2020		2	030		*	204	0 *	*	2	050 *		*	2060		
ATC CAC	TTA Leu	ATA	TA'	r CC. r Pr	A GT	C AC l Th	T AA r As	T TC n Se	C GG r Gl	A GA y As	C AA p Ly	A AC	T CA	C ACA	A r>
207	0		2	080		*	2090		*	210	0 *	*	2	110	
* TGC CC Cys Pr	* A CCG o Pro	TGO Cy	c cc s Pr	A GC	A CC	T GA	A CI u Le	C CI	rG GG eu Gl	G GG .y G1	SA CC	CG TC	A GT	C TT	C e>
	2120			213	0	,	£	2140		*	215	0 *	*	216	0
CTC TT Leu Ph	C CCC	C CC	A AA	AA CO	C A	AG G	AC A(cc co	rc A'	rG A' et I	TC TO	CC CC er A	GG AG	nr Pr	T: :0>
	2	170			218	0	*	21	90		*	2200		*	
GAG G' Glu Va	rC AC al Th	A TO	GC G ys V	rG G al V	TG G al V	TG G	AC G	TG A	GC C er H	AC G	AA G	AC C	CT G ro G	AG G' lu V	TC al>
2210	4	22	20		*	2230		*	224	. O *	,	22	50 *		*
AAG T Lys P	TC AA	AC T	GG I rp I	'AC C	TG C	Asp C	GC C	TG C	GAG C	STG (CAT A	AAT (Asn A	GCC F Ala I	AAG A Jys T	.CA 'hr>
2260	:	*	227	*		*	280 *		*		*	*	230	*	
AAG C Lys F	CCG CCPro A	GG G rg G	GAG (GAG (Glu (CAG ' Gln '	TAC .	AAC Asn	AGC / Ser	ACG '	TAC (CGT Arg	GTG (Val	GTC . Val	AGC (Ser \	GTC Val>
*	310		*	232	*	*		30		*	340		*	235	*
CTC I	ACC G	TC (CTG Leu	CAC His	CAG Gln	GAC Asp	TGG Trp	CTG Leu	TAA naA	Gly GGC	AAG Lys	GAG Glu	TAC Tyr	AAG Lys	TGC Cys>
	236				370			238				390			400

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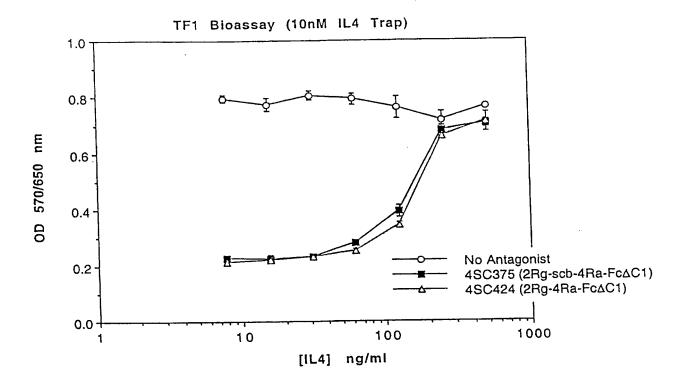
Figure 26E

	•		~		-	-										
7	١AG	GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC
I	Jys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser>
			241	0		2.4	20		2	430			244	10		
		*		*	*		*		*	*		*		*	*	
i	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA
]	ГЛS	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro>
24	Ε Λ			2460			247	7.0		2.	480			2490		
24	*		*	*		4	24	*	*	_	*		*	*		*
	TCC	CGG	GAG	GAG	ΛTG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC
	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val>
	250	20		21	510			2520			25	3.0		2	540	
	250	*	*	2:	*		*	*		*	23	*	*		*	
	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC	TAA	GGG
	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly>
	,	2550			25	60		2	570			2580			25	90
	*	233U *		*	23	*	*		*		*	*		*		*
	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG	CCI	, ccc	GTG	CTG	GAC	TCC	GAC
	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp>
		2	600			2610	ı		2.6	20		2	630			2640
	*		*		*	*		*		*	•		*		*	*
	GGC	TCC	TTC	TTC	CTC	TAT	' AGC	: AAC	CTC	ACC	GTC	GAC	AAC	3 AGC	AGC	TGG
	Gly	Ser	Phe	Phe	Lev	і Туг	Ser	Lys	Lev	ı Thi	· Val	L Asp) Lys	s Ser	Arg	Trp>
			26	550		2	2660			2670)		26	580		
		*		*	,	*	*		*		*	*		*	4	+
	CAG	CAG	G GG	AA E	GTO	C TTC	TC	A TGO	TCC	GT	G ATC	G CA	r GA	G GC	CTC	CAC
	Glr	ı Glı	n Gl	y Ası	ı Va	l Phe	e Sei	c Cys	s Se	r Va	l Me	t Hi	s Gl	u Ala	a Lev	ı His
2	690			2700	n		2.	710			2720			273	0	
ے	*		*		*	*		*		*	*		*		*	
			C TA	a 20		~ ~ ~ ~	2 20	C CM	~ TC	\sim	ദ സ്	ጥ ሮር	G GG	ልል ጥ	ል ጥር	Α
	AAG	- CA	CIA	r Th	G CA	G AA	S AG	C CI	C 1C	C C 2						

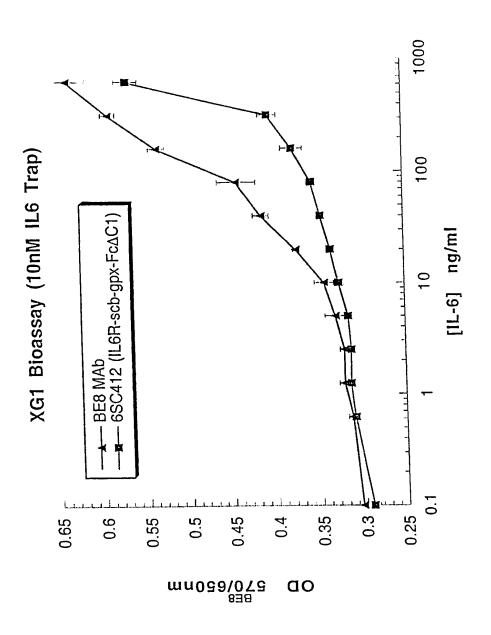
52/74 Figure 27



53/74 Figure 28



54/*7*4 Figure 29



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MRC5 Bioassay (10nM IL1 Trap) IL1 Trap 1SC569 vs IL1 Trap IL1RI.Fc

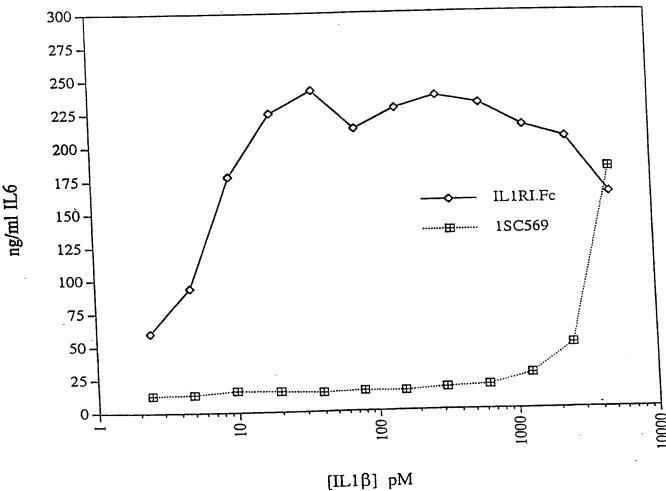


Figure 31A

10	20	30	4	0
* *	* *	* *	*	* *
ATG GTG TGG CTT				
TAC CAC ACC GAA	ACG AGA CCC G	AG GAC AAG (GGA CAC TCG	ACG GAC CAG
Met Val Trp Leu	Cys Ser Gly L	eu Leu Phe I	Pro Val Ser	Cys Leu Val>
50 60	70	8	30	90
* * *	* *	*	* *	* *
CTG CTG CAG GTG	GCA AGC TCT G	GG AAC ATG	AAG GTC TTG	CAG GAG CCC
GAC GAC GTC CAC	CGT TCG AGA C	CC TTG TAC	TTC CAG AAC	GTC CTC GGG
Leu Leu Gln Val	Ala Ser Ser G	ly Asn Met	Lys Val Leu	Gin Giu Pro>
			120	140
		20 * *	130	140
* *	* *			
ACC TGC GTC TCC				
TGG ACG CAG AGG	CTG ATG TAC T	CG TAG AGA	TGA ACG CIC	Tro Inc Mot
Thr Cys Val Ser	Asp Tyr Met S	ser lie ser	Thi Cys Giu	Tip Lys mec>
150	1.60	170	180	190
150 * * *	160	*	* *	* *
AAT GGT CCC ACC	**			TAC CAG CTG
TTA CCA GGG TGG	THE ACC TOC T	CC GAG CIC	GCG GAC AAC	ATG GTC GAC
Asn Gly Pro Thr	Acn Cvs Ser T	Thr Glu Leu	Ara Leu Leu	Tvr Gln Leu>
ASH GIY FIO III	ASII Cyb ber .	014 244		-1
200	210	220	230	240
* *	* *	* *	* *	* *
GTT TTT CTG CTC	TCC GAA GCC	CAC ACG TGT	ATC CCT GAG	AAC AAC GGA
CAA AAA GAC GAG	AGG CTT CGG (GTG TGC ACA	TAG GGA CTC	TTG TTG CCT
Val Phe Leu Leu	Ser Glu Ala I	His Thr Cys	Ile Pro Glu	Asn Asn Gly>
250	260	270		80
* *	* *	* *	*	* *
GGC GCG GGG TGC	GTG TGC CAC	CTG CTC ATG	GAT GAC GTG	GTC AGT GCG
CCG CGC CCC ACG	CAC ACG GTG	GAC GAG TAC	CTA CTG CAC	CAG TCA CGC
Gly Ala Gly Cys	s Val Cys His	Leu Leu Met	Asp Asp Val	vai Ser Ala>
		.	300	330
290 300		.0	320 * *	* *
		*		
GAT AAC TAT ACA	A CTG GAC CTG	ACC CCA CCC	CAC CAC CAC	CAC ACC TTC
Asp Asn Tyr Th	r GAC CIG GAC	Trn Ala Gly	Glo Glo Gre	Leu Tro Lvs>
Asp Asn Tyr III.	L Leu Asp Leu	IID WIG GIA	GIN GIN DO	
340	350	360	370	380
34U * *	* *	* *	*	k *
GGC TCC TTC AA	G CCC AGC GAG	CAT GTG AAA	CCC AGG GC	C CCA GGA AAC
CCG AGG AAG TT	C GGG TCG CTC	GTA CAC TTT	GGG TCC CG	G GGT CCT TTG
Gly Ser Phe Ly	s Pro Ser Glu	His Val Lys	Pro Arg Ala	a Pro Gly Asn>
		_		

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Figure 31B

					U										
	390			40	0		4	10			420			43	0
*	*		*		*	*		*		*	*		*		*
CTG	ΛCA	GTT	CAC	ACC	TAA	GTC	TCC	GAC	ACT	CTG	CTG	CTG	ACC	TGG	AGC
			GTG												
Leu	Thr	Val	His	Thr	Asn	Val	Ser	Asp	Thr	Leu	Leu	Leu	Thr	Trp	Ser>
											_				400
	4	140 *		*	450 *		*	46	· O		4	.70 *		*	480
* * * *	000		CCC			א א תי		CTC		יחתת	ሮልጥ		ACC		
			GGG												
															Ala>
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		49	90		5	00			510			52	2.0		
	*		*	*		*		*	*		*		*	*	
GTC	AAC	ATT	TGG	AGT	GAA	AAC	GAC	CCG	GCA	GAT	TTC	AGA	ATC	$\mathbf{T}\mathbf{A}\mathbf{T}$	AAC
			ACC												
Val	Asn	Ile	Trp	Ser	Glu	Asn	Asp	Pro	Ala	Asp	Phe	Arg	Ile	Tyr	Asn>
						_									
530			540 *			5	50 *	*		56°0 *		*	570		*
* CTC	200	* m>~	CTA	C 3 3	CCC.	maa			አጥሮ		CCC			CTTC	
			GAT												
															Lys>
٧۵١		- 1 -	200		110	001	200	•••							-,-
5	80			590			600			6	10	•		620	
	*	*		*		*	*		*		*	*		*	
			TCC												
															ATA
Ser	Gly	Ile	Ser	Tyr	Arg	Ala	Arg	Val	Arg	Ala	Trp	Ala	Gln	Ser	Tyr>
	c 2 0			_	4.0			CEA			660			6	70
*	630		*	ь	40 *	*		650 *		*	*		*		*
	ACC	י ארר	' тсс	AGT		TGG	AGC	CCC	AGC	ACC	AAG	TGG	CAC	: AAC	TCC
															AGG
															Ser>
			_			_									
		680			690			7	00			710			720
*		*		*	*		*		*	4	k .	*		*	*
TAC															CCT
															GGA
ТУ1	r Arg	g G11	ı Pro) Phe	e GIU	i Gli	ı Se:	c GT	GT?	Y GI	Y GIZ	A GTZ	Y AL	a Ala	a Pro>
			730			740			750	n			760	•	
	*		*		t	*		*	, , ,	*	*		*		*
ACC	G GA	A AC	r cac	G CC2	A CCI	r GT	G AC	A AA'	r T T	G AG	r GT	C TC	T GT	T GA	A AAC
															T TTG
Th	r Gl	u Th	r Gl	n Pro	o Pro	o Va	l Th	r Ası	n Le	u Se	r Va	1 Se	r Va	1 G1	u Asn>

Figure 31C

770			780		790				800				810			
*		*	*		*		*	*		*		*	*		*	
								TAA								
								TTA								
Leu	Cys	Thr	Val	Ile	Trp	Thr	Trp	Asn	Pro	Pro	GIU	GIY	Ala	ser	Ser>	
82	20		8	30			840			85	0		8	60		
	*	*		*		*	*		*		*	*		*		
								CAT								
								GTA								
Asn	Cys	Ser	Leu	Trp	Tyr	Phe	Ser	His	Phe	Gly	Asp	Lys	Gln	Asp	Lys>	
	870			88	0		8	90			900			91	.0	
*	*		*		*	*		*		*	*		*		*	
								TCA								
								AGT								
Lys	Ile	Ala	Pro	Glu	Thr	Arg	Arg	Ser	Ile	Glu	Val	Pro	Leu	Asn	Glu>	
	9	920			930			94	0		9	950			960	
*		*		*	*		*		*	*		*		*	*	
AGG	ATT	TGT	CTG	CAA	GTG	GGG	TCC	CAG	$\mathbf{T}\mathbf{G}\mathbf{T}$	AGC	ACC	AAT	GAG	AGT	GAG	
								GTC								
Arg	Ile	Суѕ	Leu	Gln	Val	Gly	Ser	Gln	Суѕ	Ser	Thr	Asn	Glu	Ser	Glu>	
		9	70		9	980			990			10	00			
	*		*	*		*		*	*		*		*	*		
								TGC								
															CTA	
Lys	Pro	Ser	Ile	Leu	Val	Glu	Lys	Cys	IIe	Ser	Pro	Pro	GIU	GIĀ	Asp>	
1010			1020			10	30		1	040			1050			
*		*	*		*		*	*		*		*	*		*	
															AGC	
GGA	CIC	AGA	CGA	CAC	TGA	CTC	GAA	GTT	ACG	TAA	ACC	GTG	TTG	GAC	TCG	
Pro	Glu	Ser	Ala	Val	Thr	Glu	Leu	Gin	Cys	tite	rrp	HIS	ASI	. Leu	Ser>	
10	60		1	070			1080			10	90		1	100		
	*	4		*		*	*		*		*	*		*		
TAC		: AAC	TGT	TCT	TGG	CTC	CCT	GGA	AGC	raa e	ACC	AG	r ccc	GAC	ACT	
አ ጠረ	ATC					CAC	GGA	CCT	TCC	TTA	Y TGG	TC	GGC	CTC	TGA	
AIC	TAC	TTC	ACA	AGA	ACC	- GAG	_		_	_	- T- 1					
Ту	TAC	TTC	ACA	AGA Ser	ACC	Leu	Pro	Gly	Arç	g Asr	Thi	Sei	Pro	Ası	Thr>	
Туг	TAC	TT(ACA	Ser	Trp	Leu	Pro	.130	Arq	g Asr	1140	Sei	r Pro	Ası	Thr>	
Ту: *	TAC Met	TTC Lys	ACA Cys	Ser 11	Trp .20 *	Leu *	Pro	.130 *	Arq	, Asr	1140	Sei	r Pro	Ası 1:	50 *	
Ту: * АА (TAC Met 1110	TTC Lys	C ACA CYS * T CTC	Ser 11	Trp .20 *	Leu * TGG	Pro 1 CAC	Gly .130 * .AGA	Arq AG	Asr *	1140	Sei	r Pro	Asr 1: CA'	Thr>	
ТУ1 * AA(ТТ(TAC Met 1110 TAC G AT	TTC Lys A TG	C ACA Cys * CTC A GAC	S Ser 11 C TAC G ATC	Trp .20 * TAT	teu * TGG	Pro 1 CAC	Gly .130 * C AGA	Arg AGC TCC	* C CTC G GAC	1140 GAZ	Sei	r Pro * A AT' r TA	ASI 1: CA' A GT	50 *	

Figure 31D

	1160 1170						1180				1190				200
*		*		*	*		*		*	*		*		*	*
TGT	GAA	AAC	ATC	TTT	AGA	GAA	GGC	CAA	TAC	TTT	GGT	TGT	TCC	TTT	GAT
											CCA				
Cys	Glu	Asn	Ile	Phe	Arg	Glu	Gly	Gln	Tyr	Phe	Gly	Cys	Ser	Phe	Asp>
		12:			12	20		*	230		*	124	*	*	
~~~	*		*	*	O 3 M	*	3 CM		~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	ממי		х Ст			מידית
CTG	ACC	AAA	GTG	AAG	GAT.	TCC	AGT.	л и и и Л.Т.Т.	CUUT	CMM	CAC GTG	TC Y	CVC	CWW	ጥልጥ
GAC	T.G.C.	THE	UAC	TYC	Acn	Sor	Ser	Phe	Glu	Gln	His	Ser	Val	Gln	Ile>
ьeu	THE	гуѕ	vaı	Lys	wsb	Ser	261	FILE	Giu	9211		ber	V W L	U = 1.1	110-
1250			1260			127	0		12	280		:	1290		
*		*	*		*		*	*		*		*	*		*
ATG	GTC	AAG	GAT	AAT	GCA	GGA	AAA	TTA	AAA	CCA	TCC	$\mathtt{TTC}$	AAT	ATA	GTG
TAC	CAG	TTC	CTA	TTA	CGT	CCT	TTT	TAA	$\mathbf{T}\mathbf{T}\mathbf{T}$	GGT	AGG	AAG	TTA	TAT	CAC
Met	Val	Lys	Asp	Asn	Ala	Gly	Lys	Ile	Lys	Pro	Ser	Phe	Asn	Ile	Val>
13			_	310			L320			13	30 *	_	13	340 *	
	*	*		*		*	*	~~ =	*	~~~		*	* * *		CmC
CCT	TTA	ACT	TCC	CGT	GTG	AAA	CCT	GAT	CCT	CCA	CAT	WILL	MAA	MAC	CAC
GGA	AAT	TGA	AGG	GCA	CAC	Thr	GGA D=0	CTA	Bro	Dro GG1	GTA	Tla	TAG	11G	Leu>
Pro	rea	unr	ser	Arg	vai	гЛS	PIO	Asp	FIO	FIO	1112	110	כעם	ASII	Deu-
	1350	,		13	60		1	370			1380			13	90
*	*		*		*	*		*.		*	*		*		*
TCC	TTC	CAC	. AAT	GAT	GAC	CTA	TAT	GTG	CAA	TGG	GAG	TAA	CCA	CAG	TAA
AGG	AAG	GTO	TTA	CTA	CTG	GAT	ATA	CAC	GTT	ACC	CTC	TTA	GGT	GTC	TTA
Ser	Phe	His	Asn	Asp	Asp	Leu	Tyr	Val	Gln	Trp	Glu	Asn	Pro	Gln	Asn>
		400			1410			14	20	y.		430		*	1440
*		*		*	* 		* 						ቦ አልሮ		CAA
1"1"1	. A.1".	r AG	: AGA	r vac	CTA	. עעע י TTT	מחע	CTTT	י כוני רמחי	י כולינ מנציג	CAC	: ጥጥ	1 TAC	TCG	GTT
Pho	1744 1744	1 100	a rea	CVC	. Len	Phe	ጥህን	Gli	. Val	Gli	val	Ası	n Asr	Ser	Gln>
FILE	. 114	= 3C.	r wr	y Cys	, DC		1-	010							_
		1	450		1	460			1470	)		1	480		
	*		*		k	*		*		k	*		*	+	•
AC	r GA	G AC	A CA	r AA	r GTI	TTC	TAC	GTO	CA	A GA	G GC	CAA 1	A TG	GAC	TAA
TG	A CT	C TG	T GT	A TT	A CA	AA A	ATC	CA	GT	r CT	C CG	A TT	T AC	CTC	ATT
Th:	r Gl	u Th	r Hi	s Ası	n Val	l Phe	э Туз	. Va	l Gl	n Gl	u Ala	a Ly	s Cy	s Glu	Asn>
			4	_			- 1 0			1 5 2 2			152	1	
1490		*	150	0	*	1:	510		*	1520 *		*	153	J ★	*
*	n ~~		m ~x	_ G %C		ኮ ርጥ					ጥ ጥር-				с сст
رر. در.	ጥ ርጥ	ъд 77 Т. Т.Т	ኔ ርጥ ል	יט אט. ר ידרי	ሲ <u>ሲ</u> ሊሲ: የ፣ የጋሚ	A CA	C CTI	inter	A TG	T AG	A AC	A AA	G TA	CA	G GGA
Pr	o G1	u Ph	e Gl	u Ar	g As:	n Va	1 G1	u As	n Th	r Se	r Cy	s Ph	e Me	t Va	l Pro>

### Figure 31E

1540 1550 * * *					1560 1570 * * * *				0 *	1580				
GGT GTT	СТТ	CCT	GAT .	ACT 1	TTG .	AAC A	ACA (	GTC	AGA TCT	ATA TAT	NGA TCT	GTC CAG	AAA TTT	ACA TGT
Gly Val	Leu	Pro	Asp	Thr	Leu	Asn '	rhr	Val	Arg	Ile	Arg	Val	Lys	Thr>
1590			160			16				620		*	163	30
* *		*		*	*	a. 0	*	ama.	*		חתת		አርር	
AAT AAG TTA TTC	TTA	TGC	TAT	GAG	GAT	GAC	MAA. TOTOTO	CAC	ACC.	MG I	ውጥ አ የረጉን	ACC	TCG	GTT
TTA TTC Asn Lys	AAT	ACG	ATA	CIC	ACD.	Acn	TAZE	Len	Trn	Ser	Asn	Trp	Ser	Gln>
Asn Lys	Leu	Cys	TÄL	GIU	MSD	ASP	БŽЗ	БСС	110	001				
_	640			.650		*	166	50 *	*	1	670 *		*	1680
GAA ATG	.*		*		3 3 C		አአጥ			<b>ACC</b>		GAC	ααα	
GAA ATG	AGT	ATA	GGT	DAA TIME	ጥጥር	CGC	ጥጥΆ	AGG	TGT	TGG	CCT	CTG	TTT	TGA
Glu Met	Ser	TAI	Gly	TAC	LVS	Ara	Asn	Ser	Thr	Thr	Gly	Asp	Lys	Thr>
GIU MEC	. Ser	110	Gry	טעב		5					_	_		
	16	90		17	700		1	1710			17	20		
*		*	*		*		*	*		*		*	*	
CAC ACA	YGC	CCA	CCG	TGC	CCA	GCA	CCT	GAA	CTC	CTG	GGG	GGA	CCG	TCA
GTG TG	ACG	GGT	GGC	ACG	GGT	CGT	GGA	CTT	GAG	GAC	CCC	CCT	GGC	AGT
His Th	Cys	Pro	Pro	Суѕ	Pro	Ala	Pro	GLu	ьeu	Leu	. Сту	GIŞ	Pro	Ser>
1770		1710			4 7	<b>-</b> 0			260			1770		
1730		1740			17			1	760					
*	*	*		*		*	*		*		*	*	•	*
* 	- <b>C</b> TC		CCC	CCA	AAA	*	AAG	GAC	*	CTC	TA	* OTA :	TCC	CGG
* GTC TTC	CTC	* TTC	CCC	CCA	AAA TTT	* CCC GGG	AAG TTC	GAC	* ACC	GAC	TAC	* OTA E	TCC AGO	CGG GCC
* 	CTC	* TTC	CCC	CCA	AAA TTT	* CCC GGG	AAG TTC	GAC	* ACC	GAC	TAC	* OTA E	TCC AGO	CGG GCC
* GTC TTC CAG AA Val Ph	CTC	* TTC AAG Phe	CCC GGG Pro	CCA	AAA TTT	* CCC GGG	AAG TTC Lys	GAC	* ACC TGG	GAC	TAC	* G ATC TAC	TCC AGO	CGG GCC
GTC TTC CAG AA Val Ph	CTC GGAG e Lev	* TTC AAG Phe	CCC GGG Pro	CCA GGT Pro	AAA TTT Lys	CCC GGG Pro	AAG TTC Lys	GAC CTG Asp	* ACC TGG Thr	G GAC Lev 310	TAC TAC Met	* ATC TAC : Ile	TCC G AGC e Ser L820	C CGG G GCC C Arg>
GTC TTC CAG AA Val Ph	CTC GGAG e Leu *	* TTC AAG Phe	CCC GGG Pro .790	CCA GGT Pro	AAA TTT Lys *	CCC GGG Pro 1800	AAG TTC Lys	GAC CTG Asp *	* ACC TGG Thr	G GAG Lev B10 *	TAC TAC Met	* ATO	TCC F AGC F Ser L820	C CGG G GCC Arg>
TGG GG	C CTC G GAG e Leu T GAG	* TTC AAG Phe 1 GGTC	CCC GGG Pro .790 *	CCA GGT Pro	AAA TTT Lys *	CCC GGG Pro 1800	AAG TTC Lys	GAC CTG Asp *	* ACC TGG Thr  18 C GTC	G GAG Lev 310 * G AGG	C CAC	* G ATO C TAG C Ile  1 A C GAA G CT	C TCC G AGC E Sei 1820 * A GAC	C CGG G GCC Arg> C CCT G GGA
GTC TTC CAG AA Val Ph	C CTC G GAG e Leu T GAG	* TTC AAG Phe 1 GGTC	CCC GGG Pro .790 *	CCA GGT Pro	AAA TTT Lys *	CCC GGG Pro 1800	AAG TTC Lys	GAC CTG Asp *	* ACC TGG Thr  18 C GTC	G GAG Lev 310 * G AGG	C CAC	* G ATO C TAG C Ile  1 A C GAA G CT	C TCC G AGC E Sei 1820 * A GAC	C CGG G GCC Arg> C CCT G GGA
TGG GG	C CTC G GAG e Leu T GAC A CTC	* TTC AAG Phe 1 GGTC	CCC GGG Pro .790 * C ACA	CCA GGT Pro	AAA TTT Lys *	CCC GGG Pro 1800 * GGTG CAC	AAG TTC Lys GTG CAC	GAC CTG Asp *	* ACC TGG Thr  18 C GTC	G AGC TCC	C CAGG GTG	* G ATO C TAG C Ile  1 A C GAA G CT	C TCC G AGC E Ser L820 * A GAC T CTC	C CGG G GCC Arg> C CCT G GGA p Pro>
TGG GG	C CTC G GAG e Leu T GAC A CTC	* TTC AAG Phe 1 G GTC CAG	CCC GGG Pro .790 * C ACA	CCA GGT Pro A TGC T ACG C Cys	AAA TTT Lys  * GTG CAC	CCC GGG Pro 1800 * G GTG C CAC	AAG TTC Lys	GAC CTG Asp *	* ACC TGG Thr  18 C GTC	G GAGG AGG TCG 186	C CAGG GTG	* G ATO C TAG C Ile  1 A C GAA G CT	C TCC G AGC E Ser 1820 * A GAC T CTC U As	C CGG G GCC Arg> C CCT G GGA
TGG GGThr Pr	C CTC G GAG e Leu T GAC A CTC	* TTC AAG Phe 1 C CAG Val	CCC GGG Pro .790 * C AC# TGT L Thi	CCA GGT Pro A TGC T ACG C Cys	AAA TTT Lys * GTG CAG	CCC GGG Pro 1800 * GGTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * G GAC CTC L Asp	* ACC TGG Thr  18 C GTC C CAC	G GAGGE AGGE TOOL SEE	C CAG G THI C CAG G GTG HI	* G ATC TAC TAC TAC G GA G CT s Gl	C TCC G AGC E Ser 1820 * A GAC T CTC U As	C CGG G GCC C Arg> C CCT G GGA p Pro>
TO TTO CAG AAC Val Photo ACC CC TGG GG Thr Proceed TGG GG Thr Proceed TGG GG TGG GG TGG GG TGG GG TGG GG TGG GG	C CTC G GAG e Leu T GAG A CTC O Glv	* TTC G AAG A Phe 1 G GTC C CAG A Val	CCC GGG Pro .790 * ACA TGT L Thi	CCA GGT Pro A TGC T ACG C Cys	AAA TTT Lys  * GTG CAG Val	* CCC GGG Pro 1800 * GGTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * GGAC CTC Asp	* ACC TGG Thr  18 C GTG C CAC	G GAGG TCG 186	C CAG G GTG G GTG T Hi	* ATC TAC TAC TAC TAC TAC TAC TAC TAC TAC	C TCC G AGC E Ser L820 * A GAC T CTC U AS	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC
TTC CTC CTC CTC CTC CTC CTC CTC CTC CTC	C CTC G GAG e Leu T GAG A CTC o Glv	* TTC G AAG I Phe C CAG I Val	CCC GGG Pro * ACA TGT L Thi	CCA GGT Pro A TGC T ACG Cys 840 *	AAA TTT Lys  * CAC Val	CCC GGG Pro 1800 * GGTG CAC Val  * CGTG GCAC	AAG TTC Lys GTG CAC	GAC CTG Asp * G GAC C CTC L Asp	* CGTGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGCAG	G GAGC TCC1 Sec. 186	C CACG GTC Hi	* G ATC C TAC E Ile  * C GAA G CT S GI * C CA C	C TCC G AGC E Ser L820 * A GAC T CTC U AS 1 T AA	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC CA CGG
TTC CTC CTC CTC CTC CTC CTC CTC CTC CTC	T GACA CTC AAAG TT	* TTC G AAG A Phe G GTC C CAG U Val  * G TTC C AAC S Phe	CCC GGG Pro * ACA TGT L Thi	CCA GGT Pro A TGC T ACG C Cys 840 * C TGC G ACC	AAA TTT Lys  * GTG CAC TAC TAC TY	CCC GGG Pro 1800 * GGTG CAC Val  * CGTG GCAC	AAG TTC Lys GTG CAC Val .850 * GAC CTG Asj	GAC CTG Asp * GGAC CTC Asp CGGC GGC	* CGTGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGCAGGCAG	G GAGC TCC1 Sec. 186	C CACG GTC Hi	* TAG C GAA C GAA C GCA	C TCC G AGC E Ser L820 * A GAC T CTC U AS 1 T AA	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC
TGG GGThr Pr	T GAGA CTG	* TTC AAG Phe 1 C CAG Val	CCC GGG Pro .790 * C ACA G TGT L Thi	CCA GGT Pro A TGC T ACG C Cys 840 * C TGC G ACC In Tri	AAA TTT Lys  * GTG G CAC G Val	CCC GGG Pro 1800 * GGTG CAC Val * CGTCG CAC * *	AAG TTC Lys GTG CAC Val .850 * GAC CTG Ass	GAC CTG Asp * GGAC CTC Asp GCC GCC pG1	* CGTGGCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	G AGC TCC 186 G GA C TCC 186 G GA C CT 1 G1	C CAG G GT G GT Hi C CA C CA C CA Va 1910	* GATO C TAG C TAG C GATO S GI C GATO	C TCC G AGC E Ser L820 A GAG T CTC U AS 1 T AA T AA	C CGG G GCC Arg> C CCT G GGA p Pro> 870 * T GCC A CGG Sn Ala> 1920 *
TGG GG Thr Pr  183 * GAG GT CTC CAG TGG GG Thr Pr  183 * GAG GT CTC CAG Glu Va	T GAGA CTG	* TTC AAG Phe 1 CAG CAG CAG CAG	CCC GGG Pro .790 * CACA GTGT LThi	CCA GGT Pro A TGC T ACG C Cys B40 * C TGC G ACC IN Tri	AAA TTT Lys  * GTG G CAC G TAC G TY  O Ty  G GA	CCC GGG Pro 1800 * GGTG CAC Val * CGTCG CAC * CGTCG CGTCG CGC CAC * CG	AAG TTC Lys GTG CAC Val .850 * GAC CTG As;	GAC CTG Asp * GGAC CTC Asp C GG G CC p G1	* CGTGGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA	G AGGC TCGL Se CTGL GAGGC GAGCC GAGGC GAGCC GACCC GACC	C CAG G GT G GT C CA G GT Hi C CA 1910	* GATO TAG	C TCC G AGC E Ser L820 A GAC T CTC U AS 1 T AA TAA TAA TAA TAA TAA TAA TAA TAA TA	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC CA CGG sin Ala> * 1920 * TG GTC
TTC TTC	T GAGA CTG AAAG TT 1880	* TTC AAG Phe 1 C CAG C C AAG S Ph	CCC GGG Pro .790 * CACA GTGI LThi 18 CAA( GTTG e As:	CCA GGT Pro A TGC T ACG C Cys B40 * C TGC G ACC IN Tri	AAA TTT Lys  * GTG CAC TAC TY  TY  GGA CCC	CCC GGG Pro 1800 * GGTG CAC Val * CGTCG CAC * CGTCG CAC CGTCG CAC CGTCG CAC CGTCG CAC CGTCG CGTC	AAG TTC Lys GTG CAC Val .850 * GAC CTG As;	GAC CTG Asp  * GGAC CTC Asp CGGC GCC PGI * CAA	* CGTGGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA	G AGC TC G GAGC TC G GAGC G GAGC G GAGC G GAGC G GG	C CAG G GT G GT C CA G GT C CA U Va 1910	* ATO C TAG C TAG C TAG C GA C	C TCC G AGC E Ser L820 A GAC T CTC U AS: 1 T AA TAA TA TT S AS	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC CA CGG Sin Ala> * 1920 * TG GTC AC CAG
TTC TTC	T GAGA CTG AAAG TT 1880	* TTC AAG Phe 1 C CAG C C AAG S Ph	CCC GGG Pro .790 * CACA GTGI LThi 18 CAA( GTTG e As:	CCA GGT Pro A TGC T ACG C Cys B40 * C TGC G ACC IN Tri	AAA TTT Lys  * GTG CAC TAC TY  TY  GGA CCC	CCC GGG Pro 1800 * GGTG CAC Val * CGTCG CAC * CGTCG CAC CGTCG CAC CGTCG CAC CGTCG CAC CGTCG CGTC	AAG TTC Lys GTG CAC Val .850 * GAC CTG As;	GAC CTG Asp  * GGAC CTC Asp CGGC GCC PGI * CAA	* CGTGGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA	G AGC TC G GAGC TC G GAGC G GAGC G GAGC G GAGC G GG	C CAG G GT G GT C CA G GT C CA U Va 1910	* ATO C TAG C TAG C TAG C GA C	C TCC G AGC E Ser L820 A GAC T CTC U AS: 1 T AA TAA TA TT S AS	C CGG G GCC C Arg> C CCT G GGA p Pro> 870 * T GCC CA CGG sin Ala> * 1920 * TG GTC

# Figure 31F

1930					19	40		1950				196				
		*		*	*		*		*	*		*		*	*	
	AGC	GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	TAA	GGC	AAG	GAG	TAC
	TCG	CAG	GAG	TGG	CAG	GAC	GTG	GTC	CTG	ACC	GAC	ATT	CCG	TTC	CTC	ATG
	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr>
19	70		1	980			199	0		20	00		2	010		
	*		*	*		*		*	*	•	*		*	*		*
	AAG	TGC	AAG	GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC
	TTC	ACG	TTC	CAG	AGG	TTG	TTT	CGG	GAG	GGT	CGG	GGG	TAG	CTC	TTT	TGG
	Lys	CA2	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr>
	202	20		20	30		2	040			205	0		2	060	
		*	. *		*		*	*		*		*	*		*	
								CCC								
	TAG	AGG	$\mathbf{T}\mathbf{T}\mathbf{T}$	CGG	TTT	CCC	GTC	GGG	GCT	CTT	GGT	GTC	CAC	ATG	TGG	GAC
	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu>
	:	2070			208	30		20	90		2	2100			21	10
	*	*		*		*	*		* .		*	*		*		*
	CCC	CCA	TCC	CGG	GAG	GAG	ATG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC
	GGG	GGT	AGG	GCC	CTC	CTC	TAC	TGG	TTC	TTG	GTC	CAG	TCG	GAC	TGG	ACG
	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	GIn	Val	Ser	ьeu	rnr	Cys>
		2	120			2130			21	40		2	150			2160
	*	2	120 *		*	2130		*	21	40 *	*	2	150 *		*	2160
	* CTG	GTC	* AAA	GGC	* TTC	* TAT	ccc	* AGC	GAC	* ATC	* GCC	GTG	* GAG	TGG	GAG	* G AGC
	GAC	GTC CAG	* : AAA : TTT	CCG	* TTC AAG	* TAT ATA	GGG	TCG	GAC CTG	* ATC TAG	CGG	GTG CAC	* GAG	ACC	GAG	* G AGC C TCG
	GAC	GTC CAG	* : AAA : TTT	CCG	* TTC AAG	* TAT ATA	GGG	TCG	GAC CTG	* ATC TAG	CGG	GTG CAC	* GAG	ACC	GAG	* G AGC
	GAC	GTC CAG	* AAA TTT Lys	CCG	* TTC AAG	* TAT ATA Tyr	GGG	TCG	GAC CTG	* ATC TAG	CGG Ala	GTG CAC	* GAG CTC	ACC	GAG	* G AGC C TCG
	GAC Leu	GTC CAG Val	* AAA TTT Lys	CCG Gly 70 *	* TTC AAG Phe	* TAT ATA Tyr	GGG Pro 180	TCG Ser	GAC CTG Asp	* ATC TAG Ile 2190	CGG Ala	GTG CAC Val	GAG CTC Glu	ACC Trr :00	GAG CTC	* G AGC C TCG I Ser>
	GAC Leu AAT	GTC CAG Val	* AAA TTT Lys 21	CCG Gly 70 *	* TTC AAG Phe	TAT ATA Tyr 2	GGG Pro 180 *	TCG Ser	GAC CTG Asp *	* ATC TAG Ile 2190 *	CGG Ala	GTG CAC Val	GAG CTC Glu	ACC Trp :00 *	GAG CTC Glu	* G AGC TCG Ser> G GAC
	GAC Leu AAT TTA	GTC CAG Val *	* AAA TTT Lys 21 G CAG	CCG Gly 70 * CCG	* TTC AAG Phe	TAT ATA Tyr  2 AAC	GGG Pro 180 * AAC	TCG Ser TAC	GAC CTG Asp * AAG	ATC TAG Ile 2190 * ACC TGG	CGG Ala ACG TGC	GTG CAC Val *	GAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	ACC Trr :00 * GTC	GAG CTC CTC CTC	* AGC TCG Ser>
	GAC Leu AAT TTA	GTC CAG Val *	* AAA TTT Lys 21 G CAG	CCG Gly 70 * CCG	* TTC AAG Phe	TAT ATA Tyr  2 AAC	GGG Pro 180 * AAC	TCG Ser TAC	GAC CTG Asp * AAG	ATC TAG Ile 2190 * ACC TGG	CGG Ala ACG TGC	GTG CAC Val *	GAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	ACC Trr :00 * GTC	GAG CTC CTC CTC	* G AGC TCG Ser> G GAC
. 2	GAC Leu AAT TTA	GTC CAG Val *	* AAA TTT Lys 21 G CAG	CCG Gly 70 * CCG	* TTC AAG Phe  * GAG CTC GIU	TAT ATA Tyr  2 AAC	GGG Pro 180 * AAC TTG	TCG Ser TAC	GAC CTG Asp * AAG	ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG TGC Thr	GTG CAC Val *	GAG Glu 22 C CCC A GGC D Pro	OO * CGTCGCACC	GAG CTC Glu	* AGC TCG Ser>
. 2	GAC Leu AAT TTA Asr	GTC CAG Val * * GGC 1 CCC	* AAA TTT Lys 21 G CAG G GTC G GTC	CCG Gly 70 * CCG GGC Pro	* TTC AAG Phe  * GAG CTC GIU	* TAT ATA Tyr 2 AAC TTG ASn	GGG Pro 180 * AAC TTG Asr	TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys	ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG TGC Thr	GTG CAC Val * CCT GGP	GAG CTC Glu 22 CCC GGG Pro	OO TTE	G GAG CTC G Glu G CTC G GAG Lev	* G AGC G Ser> G GAC C CTG L Asp>
. 2	AAT TTA Asr 210 *	GTC CAG Val * * GGG A CCC A Gly	* AAA TTT Lys 21 G CAG G GTC G GTC	CCG Gly 70 * CCG CGGC 2220 * CTCC	TTC AAG Phe  * GAG CTC Glu	TAT ATA Tyr  2 AAC TTG ASn  *	GGG Pro 180 * AAC Asr 22	TCG Ser TAC ATG TYr	GAC CTG Asp * AAG TTC Lys	* ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG TGC Thr 2240 *	GTG CAC Val * CCT GGA Pro	GAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	ACC Trr :00 * C GTC G CAC D Va: 225	G GAG C CTC G GAG C GAG Lev	* G AGC G GAC C CTG ASp>  * G AGC
. 2	AAT TTA Asr 210 * TCC	GTC CAG Val * ' GGC ' CCC ' Gly	* AAA TTT Lys 21 G CAG G GTG G GTG	CCG Gly 70 * CCG: GGC Pro 2220 TCG AGG	* TTC AAG Phe  * GAG CTC GAG TTC AAC	* TAT ATA Tyr  2 AAC TTG ASn  *	GGG Pro 180 * AAC TTG Asr 22 CTG GAG	TCG Ser TAC ATG Tyr 230 *	GAC CTG Asp * AAG TTC Lys	* ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG Thr C240 * G CTC C GAC	GTG CAC Val * CCT GGA Pro	* GTG CAG	ACC Tri	G GAG C CTC C GAG Lev C AAG	* G AGC C TTG ASP>  * G AGC C TTG C TTG C TTG C TTG
2	AAT TTA Asr 210 * TCC	GTC CAG Val * ' GGC ' CCC ' Gly	* AAA TTT Lys 21 G CAG G GTG G GTG	CCG Gly 70 * CCG: GGC Pro 2220 TCG AGG	* TTC AAG Phe  * GAG CTC GAG TTC AAC	* TAT ATA Tyr  2 AAC TTG ASn  *	GGG Pro 180 * AAC TTG Asr 22 CTG GAG	TCG Ser TAC ATG Tyr 230 *	GAC CTG Asp * AAG TTC Lys	* ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG Thr C240 * G CTC C GAC	GTG CAC Val * CCT GGA Pro	* GTG CAG	ACC Tri	G GAG C CTC C GAG Lev C AAG	* G AGC G GAC C CTG ASp>  * G AGC
. 2	AAT TTA Asr 210 * TCC AGC Second	GTC CAG Val * ' GGC ' CCC ' Gly	* AAA TTT Lys 21 G CAG G GTG G GTG	CCG Gly 70 * G CCG Pro 2220 TCG AGG Y Sei	* TTC AAG Phe  * GAG CTC GAG TTC AAC	* TAT ATA Tyr  2 AAC TTG ASn  *	GGG Pro 180 * AAC TTG Asr 22 CTG GAG	TCG Ser TAC ATG Tyr 230 *	GAC CTG Asp  * AAG TTG AGG TGG TGG TGG	* ATC TAG Ile 2190 * ACC TGG Thr	CGG Ala ACG TGC Thr C240 * CGCGAC	GTG CAC Val * CCT GGA Pro	* GTG CAG	ACC Tri	G GAG C CTC C GAG Lev C AAG	* G AGC C TTG G GAC C TTG L Asp>  # G AGC C TCG S Ser>
2	AAT TTA AST TCC AGC Sec	GTC CAG Val * GGG CCC GGT CTC CAS	* AAA TTT Lys 21 G CAG G GTC G GGC G GCC G GCC G GCC G GCC	CCG Gly 70 * G CCG G GGC 1 Pro 2220 TCG G AGG y Ser	* TTC AAG Phe  * GAG CTTC GAAC Phe 2270	* TAT ATA TYr  2 AAC TTG ASS TTG ASS	GGG Pro 180 * AAC TTG Asr 22 CTG GAG *	TCG Ser TAC ATG Tyr 30 * TATG ATA 1 Tyr	GAC CTG Asp * AAG TTC Lys	* ATC TAG Ile 2190  * ACC TGG Thr C AAC T TY	CGG Ala ACG TGC Thr C240 * G CTC G GACG Let	GTG CAC Val * CCT GGA Pro	* GAG CTC Glu 22 CCC A GGC Pro * C GTC C CAC	C GTCG CAC	G GAG G CTC G GAG Lev C AA G TT P Ly 2300	* G AGC C TTG G GAC C CTG L Asp>  * G AGC C TCG S Ser>
. 2	AAT TTA AST  * TCC AGC Sectors AGC	GTC CAG Val * GGG A CCC A GTG C AS	* AAA TTT Lys 21 G CAG G GTC G GCG G GCG G GCG	CCG Gly 70 * G CCG C GGC 2220 TCC G AGG Y Ser	* TTC AAG Phe  * GAG CTTC GAAC TTC AAC TTC GAAC	* TAT ATA TYr  2 AAC TTG ASS TTG ASS	GGG Pro 180 * AAC TTG Asr 22 CTG GAC * Let	TCG Ser TAC ATG TYr 30 * TATG ATA 1 TYr 2280	GAC CTG Asp  * AAG TTC AGC TCC CTC	* ATC TAG Ile 2190 * ACC TGG Thr C AACG T TYS	CGG Ala ACG TGC Thr CAG GCTCC CCTCC	GTG CACC GGGA TGC Thi	* GAG CTC Glu 22 CCC A GGC Pro  * CGTC CCA CCC CA CGTC CCA CGTC CGTC CGTC	C GTC C GTC C GTC C CAC C CAC C CT C CT 1 As	G GAG G CTC G GAG Lev C AA G TT P Ly 2300	* G AGC C CTG L Asp>  * G AGC C TCG S Ser>
. 2	AAT TTA AST  * TCC AGC Section 1	GTC CAG Val * GGG GCTG TAS	* AAA TTT Lys 21 G CAG G GTC G GCC G	CCG Gly 70 * GCG GCG Pro 2220 TCG AGG Y Sei * G CAG	* TTC AAG Phe  * GAG CTTC AAG CTTC	TAT ATA TYT  2 AAC TTG ASS AC TTG ASS TTG AC	GGG Pro  180  * AAC TTG Asr  22 CTG GAC  * CGTG GCA	TCG Ser Ser TAC ATG TYr 230 * TATG ATA 2280 C TTCG	GAC CTG Asp  * AAG TTC TCG AGC TCA	* ATC TAG Ile 2190 * ACC TAG Thr AAC TAG	CGG Ala ACG TGC Thr CGGAC GGAC GGAC GGAC GGAC GGAC GGAC GG	GTG CACC Val  * CCT GGA Pro CACC TGC Thi	* GAG CTC Glu 22 CCC GGC Pro  * CGTC CCA GGC Pro  * CGTC CTA	ACC Trr :000 C GTC G CAC 225 G GA C CT 1 As *	G GAG C CTC C GAC Lev C AA G TT P Ly 23000 * T GA	* G AGC C TTG G GAC C CTG L Asp>  * G AGC C TCG S Ser>

Figure 31G

TGA ACT ***>

# Figure 32A

			3	0			20			30			4	0		
7	TG	* GTG	TGG	* CCG	* GCG	CGG	* CTC	TGC	* GGG	TG.	TGG	* GCG	CTG		CTC	TGC
7	'AC	CAC	ACC	GGC	CGC	GCC	GAG	ACG	CCC	GAC	ACC	CGC	GAC	GAC	GAG	ACG
ľ	let	Val	Trp	Pro	Ala	Arg	Leu	Суѕ	Gly	Leu	Trp	Ala	Leu	Leu	Leu	Cys>
5	0			60			7	0			80			90		
	*		*	*		*	222	*	*	000	*	CCM	*	* ~~~	NOM	*
(	SCC SCC	GGC	GGC	GGG	GGC CCG	CCC	CCG	CCC	CCG	CGG	CGC	GGA	TGC	CTT	TGA	GTC
																Gln>
	10	00			110			120			1	30		1	.40	
		*	*		*		*	*		*		*	*		*	
	CCA	CCT	GTG	ACA	TAA	TTG	AGT	GTC	TCT	GTT	GAA	AAC	CTC	TGC	ACA	GTA
1	GGT Pro	GGA Pro	Val	TGT	TTA Asn	Leu	Ser	Val	Ser	Val	Glu	Asn	Leu	Cys	Thr	Val>
									170			180				90
	*	150 *		*	1	60 *	*	•	*		*	*		*		*
	ATA	TGG	ACA	TGG	AAT	CCA	CCC	GAG	GGA	GCC	AGC	TCA	TAA	TGT	AGT	CTA
	TAT	ACC	TGI	ACC	ATT	GGT	GGG	CTC	CCT	CGG	TCG	AGT	ATT	ACA	TCA	GAT Leu>
	TTE	Trp	Thr	Trp	ASN	Pro	PIO	GIU	GTĀ	AIG	. Ser	361	ASII	Cys	Der	neu-
		-	200			210		*	2	20	*		230		*	240
	* ТСС		<b>ጥ</b> ጥሳ *	ר אכיו	* CAT		GGC		AAA					ATA	GCT	CCG
	ACC	ATA	AAA	A TCA	A GTA	AAA	CCG	CTG	TTT	GT7	CTA	YTTC	TTT	TAT	CGA	GGC
	Trp	ТУ	Phe	e Ser	r His	Phe	Gly	Asp	Lys	Glr	a Asr	) Lys	Lys	Ile	Ala	Pro>
			:	250			260			270			2	280		
	C2 2	*	n . C.C.	* Tr. (C) (C)	י ייירי זי		* 	CT2	* \		* באבי	* )45) 1	AG(		r TGT	CTG
	CTI	TG	A GC.	A GC	A AG	TAT	CTI	CAI	GGC	GAG	CTT	A CTO	TC	AT C	AC	A GAC
	Glu	ı Thi	c Ar	g Ar	g Se	r Ile	e Glu	ı Val	L Pro	Le	u Ası	n Gl	ı Ar	g Ile	е Суя	s Leu>
	290			30	0		3	310			320			330	)	
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	CAZ	A GT	G GG	G TC	C CA	G TG' C AC	r AGO A TCO	C ACC	C AA' G TT	r GA A CT	G AG C TC	T GA A CT	C TT	C GG	A TC	C ATT G TAA
	Gl	n Va	1 G1	y Se	r Gl	n Cy	s Se	r Th	r As	n Gl	u Se	r Gl	u Ly	s Pr	o Se	r Ile>
		340			350			36	0			370			380	
		*		*	*		*		*	*		*	m ~~	*	*	m 00m
	TT	G GT	T GA	AA AA	LA TG	C AT	C TC.	A CC ጥ ርር	G GG	A GA	A GG	T GA	A GC	T GA	G TC C AG	T GCT A CGA
	Le	u Va	.1 G	lu Ly	rs Cy	s Il	e Se	r Pr	o Pr	o G1	u Gl	.у As	p Pr	o Gl	u Se	r Ala>

Figure 32B

390	400	)	410		420	430		
* *		* *	*	*	*	* *		
GTG ACT GAG	CTT CAA T	rgc att	rgg CAC	ANC CTG	AGC TAC	ATG AAG TGT		
CAC TGA CTC	GAA GTT A	ACG TAA	ACC GTG	TTG GAC	TCG ATG	TAC TTC ACA		
Val Thr Glu	Leu Gln (	Cys Ile '	Trp His	Asn Leu	Ser Tyr	Met Lys Cys>		
440	,	450	4.6	0	470	480		
* *	*	*	*	* *	*	* *		
TCT TGG CTC	CCT GGA	AGG AAT	ACC AGT	CCC GAC	ACT AAC	TAT ACT CTC		
AGA ACC GAG	GGA CCT	TCC TTA	TGG TCA	GGG CTG	TGA TTG	ATA TGA GAG		
Ser Trp Leu	Pro Gly	Arg Asn	Thr Ser	Pro Asp	Thr Asn	Tyr Thr Leu>		
_				<b>510</b>	-			
*	90	500 *	*	510	*	20		
						GAA AAC ATC		
ATC ATA ACC	GTG TCT	TCG GAC	CTT TTT	TAA GTA	GTT ACA	CTT TTG TAG		
Tyr Tyr Trp	His Arg	Ser Leu	Glu Lys	Ile His	Gln Cys	Glu Asn Ile>		
	_							
530	540	55		560		570		
* *	*	*	* *	*	*	* *		
TTT AGA GAA	GGC CAA	TAC TTT	GGT TGT	TCC TTT	GAT CTG	ACC AAA GTG		
AAA TCT CTT	CCG GTT	ATG AAA	Cly Cyc	Ser Phe	Aen Leu	TGG TTT CAC Thr Lys Val>		
Phe Arg Giv	GIA GIU	Tyr File	GIY CYS	Der IIIc	Map Dea	ini byo var-		
580	590		600	6	10	620		
*	* *	*	*	*	* *	*		
AAG GAT TC	C AGT TTT	GAA CAA	CAC AGT	GTC CAA	ATA ATG	GTC AAG GAT		
TTC CTA AGO	TCA AAA	CTT GTT	GTG TCA	CAG GTT	TAT TAC	CAG TTC CTA		
Lys Asp Se	r Ser Phe	Glu Gln	His Ser	Val Gln	. Ile Met	Val Lys Asp>		
630	6.0	40	650		660	670		
* *	*	* *	*	*	*	* *		
AAT GCA GG	TTA AAA A	AAA CCA	TCC TTC	ATA TAA	GTG CCT	TTA ACT TCC		
TTA CGT CC	AAT TTT T	TTT GGT	AGG AAG	TAT ATT	CAC GGA	AAT TGA AGG		
Asn Ala Gl	y Lys Ile	Lys Pro	Ser Phe	Asn Ile	e Val Pro	Leu Thr Ser>		
					710	720		
680	*	690 *	*	700 * ,	710 * *	* *		
ርርጥ ርጥር አል				AAA AA	CTC TC	C TTC CAC AAT		
GCA CAC TT	T GGA CTA	GGA GGT	GTA TA	TTT TTC	GAG AG	G AAG GTG TTA		
Arg Val Ly	s Pro Asp	Pro Pro	His Ile	E Lys Ası	n Leu Se	r Phe His Asn>		
	730	740	*	750 *	•	760 * *		
*	* * * `````````````````````````````````	* * י מאא ידיפט			ር አኔጥ ጥጥ	T ATT AGC AGA		
GAT GAC CT	A TAI GIG	CAM IGG	WA DAD 6	L COM CM	a uur ij	T WILL WOO HOW		
	ראר מייע ייי	ነ ርጥጥ አቦር	י כיוויר יוויויי	A GGጥ Gጥ	C TTA AA	A TAA TCG TCT		
Asn Asn Le	AT ATA CAC	GIT ACC	CTC TT	A GGT GT n Pro Gl	C TTA AA n Asn Ph	A TAA TCG TCT e Ile Ser Arg>		

### Figure 32C

770					790				800				810		
*		*	*		*		*	*		*		*	.*		*
	-							TAA							
								ATT							
Cys	ren	Pne	Tyr	GIU	vaı	GIU	vaı	Asn	Asn	ser	GIN	THE	GIU	THE	His>
82	20		8	30			840			85			8	60	
	*	*		*		*	*		*		*	*		*	
								AAA							
								TTT							Glu>
ASII	vai	Pne	ıyı	var	GIII	GIU	ATG	пуs	Cys	GIU	ASII	FIO	GIU	FILE	G1u>
	870			88			8	390			900			9:	LO
*	*		*		*	*		*		*	*	~~~	*		*
								TTC							
								AAG							
Arg	Asn	vaı	GIU	ASII	THE	Ser	Суѕ	Pne	Met	vaı	PIO	GIY	Val	rea	Pro>
	:	920			930			94	10		9	950			960
*		*		* 4	*		*		*	*		*		*	*
								AGA							
								TCT							
Asp	Thr	Leu	Asn	Thr	Val	Arg	Ile	Arg	Val	Lys	Thr	Asn	Lys	Leu	Cys>
		9	70		!	980			990			10	-		
	*		*	*		*		*	*		*		*	*	
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Figure 32D

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Figure 32E

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## Figure 32F

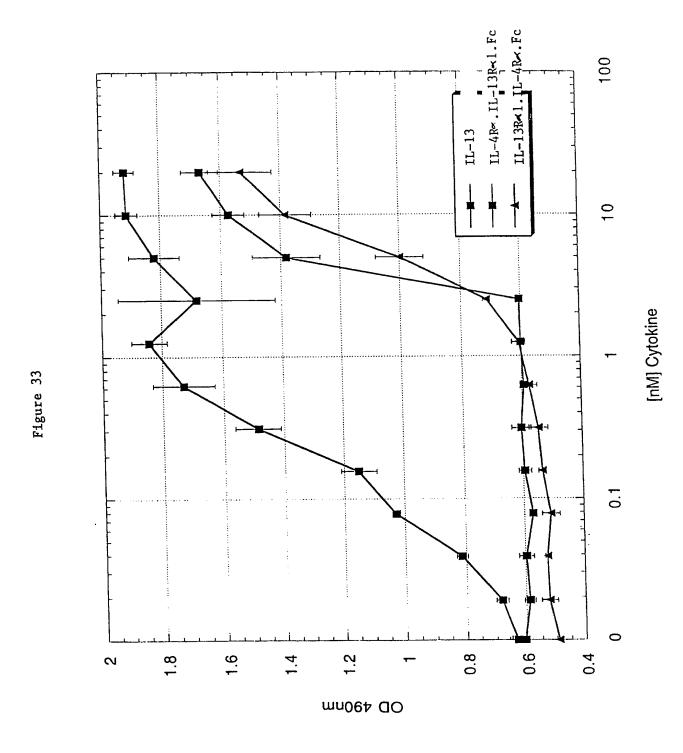
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Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His>
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Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met>
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Figure 32G

2360 2370 2380

AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>



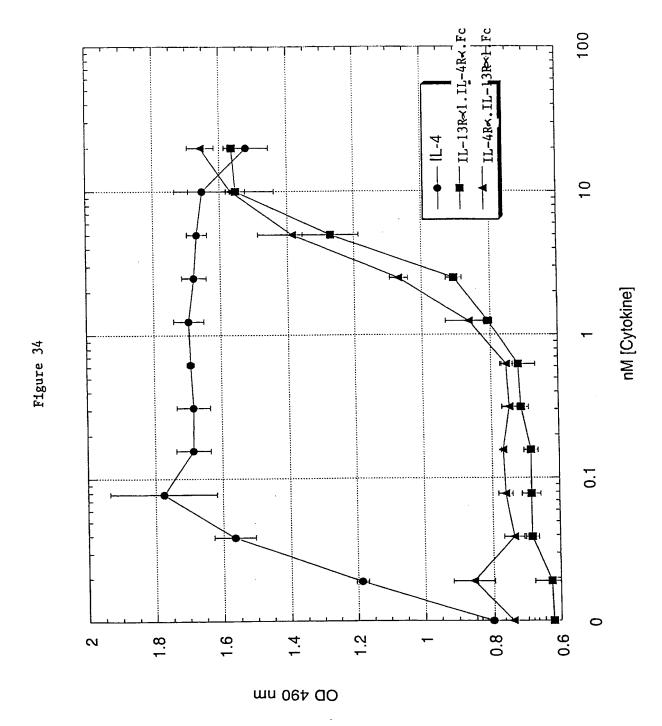


Figure 35

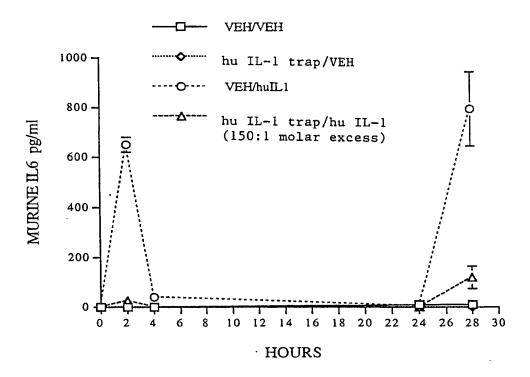


Figure 36A

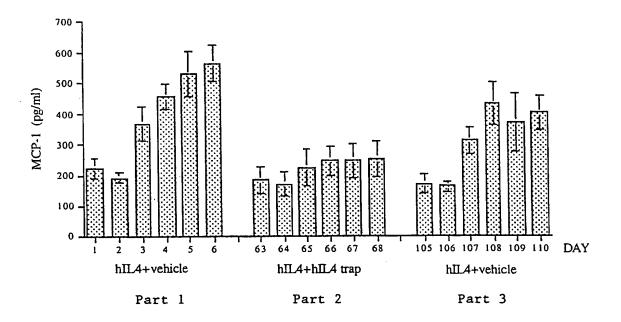


Figure 36B

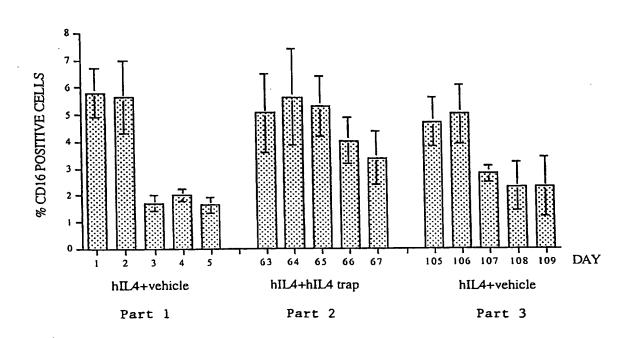
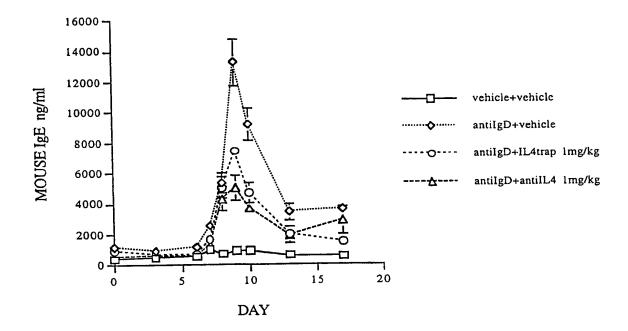


Figure 37



### **PCT**

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13,942 19 May 1999 (19.05.99) U

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- (74) Agents: SORRENTINO, Joseph, M.; Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591 (US) et al.

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#### **Published**

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(57) Abstract

The present invention provides a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex. It also provides a nucleic acid sequence encoding the fusion polypeptide and methods of making and uses for the fusion polypeptide.

*(Referred to in PCT Gazette No. 35/2000, Section II)

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DK	Denmark	LK	Sri Lanka	SE	Sweden		
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# RECEPTOR BASED ANTAGONISTS AND METHODS OF MAKING AND USING

This application claims priority of U.S. Application No. 09/313,942, filed May 19, 1999, which claims priority of U.S. Provisional Application No. 60/101,858 filed September 25, 1998. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

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### **BACKGROUND OF THE INVENTION**

Although discovered for varying biological activities, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and interleukin-6 (IL-6) comprise a defined family of cytokines (referred to 15 herein as the "CNTF family" of cytokines). These cytokines are grouped together because of their distant structural similarities [Bazan, J. Neuron 7: 197-208 (1991); Rose and Bruce, Proc. Natl. Acad. Sci. USA 88: 8641-8645 (1991)], and, perhaps more importantly, because they share "β" signal-20 transducing receptor components [Baumann, et al., J. Biol. Chem. 265:19853-19862 (1993); Davis, et al., Science 260: 1805-1808 (1993); Gearing et al., Science 255:1434-1437 (1992); Ip et al., Cell 69: 1121-1132 (1992); Stahl, et al., J. Biol. Chem. 268: 7628-7631 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Receptor activation by this family of cytokines results from 25 either homo- or hetero-dimerization of these  $\beta$  components [Davis, et al. Science 260: 1805-1808 (1993), Murakami, et al., Science 260: 1808-1810 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. IL-6 receptor activation requires homodimerization of gp130 [Murakami, et al. Science 260: 1808-1810 (1993), Hibi, et al., Cell 63: 1149-1157 (1990)], a protein initially 30 identified as the IL-6 signal transducer [Hibi, et al., Cell 63: 1149-1157 (1990)]. CNTF, LIF and OSM receptor activation results from heterodimerization between gp130 and a second gp130-related protein known as LIFR\$ [Davis,

et al., Science 260: 1805-1808 (1993)], that was initially identified by its ability to bind LIF [Gearing et al., EMBO J. 10: 2839-2848 (1991)].

In addition to the  $\beta$  components, some of these cytokines also require specificity-determining " $\alpha$ " components that are more limited in their tissue distribution than the  $\beta$  components, and thus determine the cellular targets of the particular cytokines [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus, LIF and OSM are broadly acting factors that may only require the presence of gp130 and LIFR $\beta$  on responding cells, while CNTF requires CNTFR $\alpha$  [Stahl and Yancopoulos, Cell 74: 587-590 (1993)] and IL-6 requires IL-6R $\alpha$  [Kishimoto, et al., Science 258: 593-597 (1992)]. Both CNTFR $\alpha$  (Davis et al., Science 259:1736-1739 (1993) and IL-6R $\alpha$  [Hibi, et al. Cell 63:1149-1157, Murakami, et al., Science 260:1808-1810 (1990); Taga, et al., Cell 58:573-581 (1989)] can function as soluble proteins, consistent with the notion that they do not interact with intracellular signaling molecules but that they serve to help their ligands interact with the appropriate signal transducing  $\beta$  subunits [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

Additional evidence from other cytokine systems also supports the notion that dimerization provides a common mechanism by which all cytokine receptors initiate signal transduction. Growth hormone (GH) serves as perhaps the best example in this regard. Crystallographic studies have revealed that each GH molecule contains two distinct receptor binding sites, both of which are recognized by the same binding domain in the receptor, allowing a single molecule of GH to engage two receptor molecules [de Vos, et al., Science 255: 306-312 (1992)]. Dimerization occurs sequentially, with site 1 on the GH first binding to one receptor molecule, followed by the binding of site 2 to a second receptor molecule [Fuh, et al., Science 256: 1677-1680 (1992)]. Studies with the erythropoietin (EPO) receptor are also consistent with the importance of dimerization in receptor activation, as EPO receptors can be constitutively activated by a

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single amino acid change that introduces a cysteine residue and results in disulfide-linked homodimers [Watowich, et al., Proc. Natl. Acad. Sci. USA 89:2140-2144 (1992)].

In addition to homo- or hetero-dimerization of  $\boldsymbol{\beta}$  subunits as the critical 5 step for receptor activation, a second important feature is that formation of the final receptor complex by the CNTF family of cytokines occurs through a mechanism whereby the ligand successively binds to receptor components in an ordered manner [Davis, et al. Science 260:1805-1818 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus CNTF first 10 binds to CNTFR $\alpha$ , forming a complex which then binds gp130 to form an intermediate (called here the  $\alpha\beta1$  intermediate) that is not signaling competent because it has only a single  $\beta$  component, before finally recruiting LIFR $\beta$  to form a heterodimer of  $\beta$  components which then 15 initiates signal transduction. Although a similar intermediate containing IL-6 bound to IL-6R $\alpha$  and a single molecule of gp130 has not been directly isolated, we have postulated that it does exist by analogy to its distant relative, CNTF, as well as the fact that the final active IL-6 receptor complex recruits two gp130 monomers. Altogether, these findings led to a 20 proposal for the structure of a generic cytokine receptor complex (Figure 1) in which each cytokine can have up to 3 receptor binding sites: a site that binds to an optional  $\alpha$  specificity-determining component ( $\alpha$  site), a site that binds to the first  $\beta$  signal-transducing component ( $\beta$ 1 site), and a site that binds to the second  $\beta$  signal-transducing component ( $\beta$ 2 site) [Stahl 25 and Yancopoulos, Cell 74: 587-590 (1993)]. These 3 sites are used in sequential fashion, with the last step in complex formation -- resulting in β component dimerization -- critical for initiating signal transduction [Davis, et al. Science 260:1805-1818 (1993)]. Knowledge of the details of receptor activation and the existence of the non-functional \$1 30 intermediate for CNTF has led to the finding that CNTF is a high affinity

antagonist for IL-6 under certain circumstances, and provides the strategic basis for designing ligand or receptor-based antagonists for the CNTF family of cytokines as detailed below.

5 Once cytokine binding induces receptor complex formation, the dimerization of  $\beta$  components activates intracellular tyrosine kinase activity that results in phosphorylation of a wide variety of substrates [Ip, et al. Cell 69:121-1132 (1992)]. This activation of tyrosine kinase appears to be critical for downstream events since inhibitors that block the tyrosine 10 phosphorylations also prevent later events such as gene inductions [Ip, et al., Cell 69:121-1132 (1992); Nakajima and Wall, Mol. Cell. Biol. 11:1409-1418 (1991)]. Recently, we have demonstrated that a newly discovered family of non-receptor tyrosine kinases that includes Jak1, Jak2, and Tyk2 (referred to as the Jak/Tyk kinases) [Firmbach-Kraft, et al., Oncogene 15 5:1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11: 2057-2065 (1991] and that are involved in signal transduction with other cytokines [Argetsinger, et al., Cell 74:237-244 (1993); Silvennoinen, et al., Proc. Natl. Acad. Sci. USA 90:8429-8433 (1993); Velazquez, et al., Cell 70: 313-322 (1992); Witthuhn, et al., Cell 74:227-236 (1993)], preassociate with the cytoplasmic domains of the 20 β subunits gp130 and LIFRβ in the absence of ligand, and become tyrosine phosphorylated and activated upon ligand addition [Stahl et al., Science 263:92-95 (1994)]. Therefore these kinases appear to be the most proximal step of intracellular signal transduction activated inside the cell as a result of ligand binding outside of the cell. Assay systems for screening collections of small molecules for specific agonist or antagonist activities 25 based on this system are described below.

The CNTF family of cytokines play important roles in a wide variety of physiological processes that provide potential therapeutic applications for both antagonists and agonists.

### SUMMARY OF THE INVENTION

An object of the present invention is the production of cytokine antagonists that are useful in the treatment of cytokine-related diseases or disorders.

Another object of the invention is the use of the disclosed cytokine antagonists for the treatment of cytokine-related diseases or disorders. For example, an IL-6 antagonist described herein may be used for the treatment of osteoporosis, the primary and second effects of cancers, including multiple myeloma, or cachexia.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of cytokine receptors.

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Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of the cytokines.

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of members of the CNTF family of cytokines.

Another object of the invention is the development of screening systems
useful for identifying small molecules that act as agonists or antagonists of the CNTF family of cytokines.

### BRIEF DESCRIPTION OF THE FIGURES

30 FIGURE 1: Ordered binding of receptor components in a model of a generic cytokine receptor. The model indicates that cytokines contain up to 3 receptor binding sites and interact with their receptor components by

binding first the optional  $\alpha$  component, followed by binding to  $\beta 1$ , and then  $\beta 2$ . The  $\beta$  components for many cytokine receptors interact through membrane proximal regions (shaded boxes) with the Jak/Tyk family of cytoplasmic protein tyrosine kinases. Only upon dimerization of  $\beta$  components is signal transduction initiated, as schematized by the tyrosine phosphorylations (P) of the  $\beta$  components and the Jak/Tyk kinases.

FIGURE 2: CNTF inhibits IL-6 responses in a PC12 cell line (called PC12D) that expresses IL6Rα, gp130, CNTFRα, but not LIFRβ. Serum-deprived PC12D cells were incubated + IL-6 (50 ng/mL) in the presence or absence of CNTF as indicated. Some plates also received soluble IL6Rα (1 mg/mL) or soluble CNTFRα (1 mg/mL) as indicated. Cell lysates were subjected to immunoprecipitation with anti-gp130 and immunoblotted with anti-phosphotyrosine. Tyrosine phosphorylation of gp130 is indicative of IL-6 induced activation of the IL-6 receptor system, which is blocked upon coaddition of CNTF.

FIGURE 3: Scatchard analysis of iodinated CNTF binding on PC12D cells. PC12D cells were incubated with various concentrations of iodinated CNTF in the presence or absence of excess non-radioactive competitor to determine the specific binding. The figure shows a Scatchard plot of the amount of iodinated CNTF specifically bound, and gives data consistent with two binding sites with dissociation constants of 9 pM and 3.4 nM.

FIGURE 4. The amino acid sequence of human gp130-Fc-His6. Amino acids 1 to 619 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of gp130-Fc-His6 has been italicized (amino acids 1 to 22). The Ser-Gly bridge is shown in bold type (amino acids 620, 621). Amino acids 662 to 853 are from the Fc domain of human IgG1 (Lewis, et

al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 632 and 635) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. The hexahistine tag is shown in bold/italic type (amino acids 854 to 859). (•) shows the position of the STOP codon.

FIGURE 5: The amino acid sequence of human IL-6Rα-Fc. Key: Amino acids 1 to 358 are from human IL-6Rα (Yamasaki, et al., Science 241:825-828 (1988). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of IL-6Rα-Fc has been italicized (amino acids 1 to 19). The Ala-Gly bridge is shown in bold type (amino acids 359, 360). Amino acids 361 to 592 are from the Fc domain of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 371 and 374) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that link two Fc domains. (•) shows the position of the STOP codon.

FIGURE 6: The CNTF/IL-6/IL-11 receptor system. The ordered formation 20 of the hexameric signal transducing receptor complex is depicted schematically. The cytokine associates with the  $R\alpha$  component to form an obligatory cytokine • Ra complex (Kd is about 5 nM). This low affinity complex next associates with the first signal transducing component, marked  $\beta 1$ , to form a high affinity cytokine  $R\alpha \cdot \beta 1$  complex (Kd is about 25 10 pM). In the case of IL-6Rα, this component is gp130. This trimeric high affinity complex subsequently associates with another such complex. Formation of this complex results in signal transduction as it involves dimerization of two signal transducing components, marked \$1 and \$2 respectively (adapted from (Ward et al., J. Bio. Chem. 269:23286-23289 30 (1994); Stahl and Yancopoulos, J. Neurobiology 25:1454-1466 (1994); Stahl and Yancopoulos, Cell 74:587-590 (1993).

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FIGURE 7: Design of heterodimeric receptor-based ligand traps for IL-6. The heterodimeric ligand trap is comprised of two interdisulfide linked proteins, gp130-Fc and IL-6Rα-Fc. The gp130-Fc•IL-6Rα-Fc complex (upper panel) is shown to mimic the high affinity cytokine•Rα•β1 complex (lower panel). The ligand trap functions as an antagonist by sequestering IL-6 and thus rendering unavailable to interact with the native receptors on IL-6-responsive cells.

- FIGURE 8. Heteromeric immunoglobulin Heavy/Light Chain Receptor Fusions. An example of a heavy/light chain receptor fusion molecule is schematically depicted. The extracellular domain of gp130 is fused to Cγ, whereas the extracellular domain of IL-6Rα is fused to the constant region of the kappa chain (κ). The inter-chain disulfide bridges are also depicted (S-S).
  - FIGURE 9. Amino acid sequence of gp130-Cγ1. Key: Amino acids 1 to 619 are from human gp130 (Hibi, et al., Cell 63:1149-1157 (1990). Ser-Gly bridge is shown in bold type. Amino acids 662 to 651 are from the constant region of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (*) shows the position of the STOP codon.

FIGURE 10: Amino acid sequence of gp130 $\Delta$ 3fibro. Key: Amino acids 1 to 330 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Other symbols as described in Figure 9.

FIGURE 11: Amino acid sequence of J-CH1. Key: The Ser-Gly bridge is shown in bold, the J-peptide is shown in italics, the CH1 domain is underlined.

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FIGURE 12: Amino acid sequence of Cγ4. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 239 comprise the Cγ4 sequence.

FIGURE 13: Amino acid sequence of κ-domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 108 comprise the κ domain. The C-terminal cysteine (amino acid 108) is that involved in the disulfide bond of the κ domain with the CH1 domain of Cγ.

FIGURE 14: Amino acid sequence of λ-domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 106 comprise the λ domain (Cheung, et al., J. Virol. 66: 6714-6720 (1992). The C-terminal cysteine (amino acid 106) is that involved in the disulfide bond of the λ domain with the CH1 domain of Cγ.

15 FIGURE 15: Amino acid sequence of the soluble IL-6Rα domain. Key:

Amino acids 1 to 358 comprise the soluble IL-6Rα domain (Yamasaki, et al., Science 241:825-828 (1988). The Ala-Gly bridge is shown in bold type.

FIGURE 16: Amino acid sequence of the soluble IL-6Rα313 domain: Key:

20 Amino acids 1 to 313 comprise the truncated IL-6Rα domain (IL-6Rα313).

The Thr-Gly bridge is shown in bold type.

FIGURE 17: Purification of gp130-Cγ1•IL-6Rα-κ. 4% to 12% SDS-PAGE gradient gel run under non-reducing conditions. Proteins were visualized by staining with silver. Lane 1: approximately 100 ng of material purified over Protein A Sepharose (Pharmacia). Lane 2: Molecular size standards (Amersham). Lane 3: The Protein A-purified material shown here after further purification over an IL-6 affinity chromatography step. The positions of the gp130-Cγ1 dimer [(gp130-Cγ1)2], the gp130-Cγ1 dimer

associated with one IL-6Rα-κ [(gp130-Cγ1)2•(IL-6Rα-κ)1], and the gp130-Cγ1 dimer associated with two IL-6R $\alpha$ - $\kappa$  [(gp130-C $\gamma$ 1)2•(IL-6R $\alpha$ - $\kappa$ )2] are shown, as well as the sizes for the molecular size standards in kilodaltons (200, 100, and 46).

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FIGURE 18: IL-6 dissociates slowly from the ligand trap. The dissociation rate of IL-6 from a heavy/light chain receptor-based ligand trap (gp130- $C\gamma 1 \bullet IL - 6R\alpha - \kappa$ ) was compared to that obtained with the neutralizing monoclonal antibody B-E8 (BE8 MAb).

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FIGURE 19: IL-6 can induce multimerization of the ligand trap. (A) Two different ligand traps are depicted schematically and listed according to their ability to bind protein A. gp130-Fc•IL-6Rα-Fc (GF6F) binds protein A via its Fc-domains, whereas gp130-CH1•IL-6Rα-κ (G16K) does not bind to 15 protein A. (B) Anti-kappa western blotting of proteins precipitated with Protein A-Sepharose from mixtures of GF6F ± IL-6, G16K ± IL-6, or GF6F plus G16K ± IL-6, as marked.

FIGURE 20: Inhibition of IL-6-dependent XG-1 cell proliferation. XG-1 20

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cells [Zhang, et al., Blood 83:3654-3663 (1994)] were prepared for a proliferation assay by starving the cells from IL-6 for 5 hours. Assays were set up in 96-well tissue culture dishes in RPMI + 10% fetal calf serum + penicillin/streptomycin + 0.050 nM 2-mercaptoethanol + glutamine. 0.1 ml of that media was used per well. Cells were suspended at a density of 250,000 per ml at the start of the assay. 72 hours post addition of IL-6  $\pm$ ligands traps or antibodies, an MTT assay was performed as described (Panayotatos et al. Biochemistry 33:5813-5818 (1994). The different ligand traps utilized are listed.

FIGURES 21A-21D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 424 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

5 FIGURES 22A-22D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 603 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

FIGURES 23A-23D: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 622 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

FIGURE 24A-24F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 412 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

FIGURE 25A-25F: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 616 which is capable of binding the cytokine IL-6 to form a nonfunctional complex.

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FIGURE 26A-26E: Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 569 which is capable of binding the cytokine IL-1 to form a nonfunctional complex.

25 FIGURE 27: Shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.

FIGURE 28: Shows that an IL-4 trap designated 4SC375 displays

antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap
designated 4SC424 (described in Figs. 21A-21D) which is a fusion

polypeptide of IL-2R $\gamma$ -IL4R $\alpha$ -Fc $\Delta$ C1 having the IL-2R $\gamma$  component flush with the IL-4R $\alpha$  component.

FIGURE 29: Shows that the IL6 trap (6SC412 IL6R-scb-gpx-FcΔC1) described in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

FIGURE 30: Shows that the trap 1SC569 (described in Figs. 26A-26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1.

FIGURE 31A-31G: The nucleotide and encoded amino acid sequence of the IL-4Rα.IL-13Rα1.Fc single chain trap construct is set forth.

15 FIGURE 32A-32G: The nucleotide and encoded amino acid sequence of the IL-13Rα1.IL-4Rα.Fc single chain trap construct is set forth.

FIGURE 33: Blocking of IL-13 by IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM. At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 34: Blocking of IL-4 by IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM. At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%.

FIGURE 35: Human IL-1 trap blocks the in vivo effects of exogenously administered huIL-1. BALB/c mice were given subcutaneous injection of huIL-1 (0.3  $\mu$ g/kg) at time 0. Twenty-four hours prior to huIL-1 injection, the animals were pre-treated with either vehicle or 150-fold molar excess

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of huIL-1 trap. Two hours prior to sacrifice (26 hrs), the mice were rechallenged with a second injection of huIL-1 (0.3  $\mu$ g/kg, s.c.). Blood samples were collected at various time points and sera were assayed for IL-1 levels (expressed as mean +/- SEM; n=5 per group).

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FIGURE 36A & FIGURE 36B: Human IL-4 trap antagonizes the effects of human IL-4 in monkeys. Figure 36A: Cynomologus monkeys were treated in three parts as indicated. Human IL-4 (25  $\mu g/kg$ ) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Plasma was collected daily and assayed for MCP-1 levels. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.0007; Tukey-Kramer: Part 2 vs. Part 1, p,0.05; Part 2 vs. Part 3, p,0.05; Part 1 vs. Part 3, not significant.) Figure 36B: Cynomologus monkeys were treated in three parts as indicated. Human IL-4 (25  $\mu$ g/kg) was injected subcutaneously twice daily for 4 days and human IL-4 trap (8 mg/ml) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16. Results were expressed as mean +/- SEM; n=4. (ANOVA p<0.042; Tukey-Kramer: Part 2 vs. Part 1, p<0.05; Part 2 vs. Part 3 and Part 1 vs. Part 3, not significant.)

FIGURE 37: Murine IL-4 trap partially prevented IL-4-mediated IgE increase in mice. BALB/C mice injected with anti-mouse IgD
25 (100μl/mouse, s.c.) were randomly divided into 3 groups, each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Sera were collected at various time points and assayed for IgE levels. Results were expressed as mean+/-SEM (n=5 per group). (ANOVA p=0.0002; Tukey-Kramer: vehicle vs. IL-4 trap, p<0.01; vehicle vs. IL-4 antibody, p<0.001; IL-4 trap vs. IL-4 antibody, not significant).</li>

### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:

- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
- b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
  - c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

By "cytokine binding portion" what is meant is the minimal portion of the extracellular domain necessary to bind the cytokine. It is accepted by those of skill in the art that a defining characteristic of a cytokine receptor is the presence of the two fibronectin-like domains that contain canonical cysteines and of the WSXWS box (Bazan, J.F., 1990, PNAS 87: 6934-6938). Sequences encoding the extracellular domains of the binding component of the cytokine's receptor and of the signal transducing component of the cytokine's receptor may also be used to create the fusion polypeptide of the invention. Similarly, longer sequences encoding larger portions of the components of the cytokine's receptor may be used. However, it is contemplated that fragments smaller than the extracellular domain will function to bind the cytokine and therefore, the invention contemplates fusion polypeptides comprising the minimal portion of the extracellular domain necessary to bind the cytokine as the cytokine binding portion.

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The invention comprises a "specificity determining component" of a cytokine's receptor and a "signal transducing component" of the cytokine's receptor. Regardless of the nomenclature used to designate a particular component or subunit of a cytokine receptor, one skilled in the art would recognize which component or subunit of a receptor is responsible for determining the cellular target of the cytokine, and thus would know which component constitutes the "specificity determining component."

Similarly, regardless of the nomenclature used, one of skill in the art would know which component or subunit of a receptor would constitute the "signal transducing component." As used herein, the "signal transducing component" is a component of the native receptor which is not the specificity determining component and which does not bind or weakly binds the cytokine in the absence of the specificity determining component. In the native receptor, the "signal transducing component" may participate in signaling.

For example, while some cytokine receptors have components designated  $\alpha$  and  $\beta$ , the IL-4 receptor has a signal transducing component referred to as IL-2R $\gamma$ . However, regardless of what name is associated with that component, one skilled in the art would know which component of the IL-4 receptor is the signal transducing component. Thus to practice the present invention and create a high affinity trap for IL-4, one of skill in the art would create an isolated nucleic acid comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the IL-4 receptor (IL-4R $\alpha$ ); a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the IL-4 receptor (IL-2R $\gamma$ ); and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a

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multimerizing component (for example, an Fc domain of IgG) to create a high affinity trap for IL-4.

Some further examples of the receptor components that may be used to

5 prepare cytokine antagonists according to the invention are set forth in
Table 1. The Table 1 sets forth, by way of example but not by way of
limitation, some of the varied nomenclature used in the scientific
literature to describe those components which function as specificity
determining components and those which function as signal transducing

10 components of certain cytokine receptors.

 $\beta$ -receptor component (ref. 5)

TABLE

Signal transducing Component	IL-1R AcP (refs. 8, 11)	β-chain (ref. 3) β-subunit (ref. 2) γ-chain (ref. 3) IL-2Rβ (refs. 1, 10) IL-2Rγ (refs. 1, 10)	$\beta_{c}$ (ref. 1) $\beta_{-subunit}$ (ref. 2) $\beta_{-chain}$ (ref. 3) $\beta_{-receptor}$ component (ref. 5)	γ-chain (ref. 3) IL-2Rγ (ref. 1)	$\beta_{c}$ (ref. 1) $\beta_{c}$ subunit (ref. 2) $\beta_{c}$ -chain (ref. 3)
Specificity determining Component	Type I IL-1R (ref. 8)  Type II IL-1R (ref. 8)  IL-1RI (ref. 11)  IL-1RII (ref. 11)	$\alpha$ -subunit (ref. 2) $\alpha$ -chain (ref. 3) IL-2R $\alpha$ (ref. 1)	IL-3R $\alpha$ (ref. 1) $\alpha$ -subunit (ref. 2) $\alpha$ -receptor component (ref. 5)	IL-4R (ref. 1)	IL-5R $\alpha$ (ref. 1) $\alpha$ -subunit (ref. 2) $\alpha$ -receptor component (ref. 5)
Cytokine	Interleukin-1 (IL-1)	Interleukin-2 (IL-2)	Interleukin-3 (IL-3)	Interleukin-4 (IL-4)	Interleukin-5 (IL-5)

TABLE 1 (CONT'D)

Cytokine	Specificity determining Component	Signal transducing Component
Granulocyte macrophage- colony stimulating factor (GM-CSF)	$\alpha\text{-receptor}$ component (ref. 5) $\alpha\text{-subunit}$ (ref. 2) $GMR\alpha$ (refs. 1, 2)	β-receptor component (ref. 5) β-subunit (ref. 2) β-chain (ref. 3) β _c (ref. 1) GMRβ (refs. 1, 2)
Leukemia inhibitory factor (LIF)	LIFBP (ref. 1) $\alpha$ -receptor component (ref. 5)	gp130 (refs. 1, 3) β- receptor component (ref. 5)
Interleukin-11 (IL-11)	α–chain (ref. 4) NR1 (ref. 4)	gp130 (ref. 4)
Interleukin-15 (IL-15)	IL-15R $\alpha$ (ref. 10)	IL-2R $\beta$ (ref. 10) IL-2R $\gamma$ (ref. 10)
Interferon-y (IFNy)	IFN-yR (ref. 7) IFN-yR1 (ref. 7)	AF-1 (ref. 7) IFN-yR2 (ref. 7)
TGFB	Type II (refs. 6, 9)	Type I (refs. 6, 9)

Only a few of the multitude of references are cited in Table 1, and they are set forth as follows:

- 1. Sato and Miyajima, Current Opinions in Cell Biology 6: 174-179 (1994) See page 176, lines 9-16;
- 2. Miyajima, et al., Annual Review of Immunology 10: 295-331 (1992) See page 295, line 4 to page 296, line 1; page 305, last paragraph;
- 3. Kondo, et al, Science 262: 1874-1877 (1993) See page 1874, cols. 1 & 2;
- 4. Hilton, et al, EMBO Journal 13: 4765-4775 (1994) See page 4766, col.
- 10 1, lines 20 24;

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- 5. Stahl and Yancopoulos, Cell 74: 587-590 (1993) See page 587, column 2, lines 15-22;
- 6. Bassing, et al, Journal of Biological Chemistry 269: 14861-14864 (1994) See page 14861, col. 2, lines 1-9 and 21-28;
- 7. Kotenko, et al, Journal of Biological Science 270: 20915-20921 (1995) See page 20915, lines 1-5 of the abstract;
  - 8. Greenfeder, et al., Journal of Biological Chemistry 270: 13757-13765 (1995) See page 13757, col. 1, line 6 to col. 2, line 3 and col. 2, lines 10-12; page 13764, col. 2, last 3 lines and page 13765, col. 1, lines 1-7;
- 9. Lebrun and Vale, Molecular Cell Biology 17: 1682-1691 (1997) See page 1682, Abstract lines 2-6;
  - 10. Kennedy and Park, Journal of Clinical Immunology 16: 134-143 (1996) See page 134, lines 1-7 of the abstract; page 136, col 2., lines 1-5;
  - 11. Wesche, et al., Journal of Biological Chemistry 272: 7727-7731 (1997)
- 25 See page 7731, lines 20-26.

Kotenko, et al. recently identified the IL-10R2 (IL-10Rβ) chain which is reported to serve as an accessory chain that is essential for the active IL-10 receptor complex and for initiating IL-10 induced signal transduction

events (S.V. Kotenko, et al., The EMBO Journal, 1997, Vol. 16: 5894-5903).

Additional cytokines and their receptors are described in Appendix II, page
A:9 of Immunobiology, The Immune System In Health and Disease, 2nd

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In preparing the nucleic acid sequence encoding the fusion polypeptide of the invention, the first, second, and third components of the fusion polypeptide are encoded in a single strand of nucleotides which, when expressed by a host vector system, produces a monomeric species of the fusion polypeptide. The monomers thus expressed then multimerize due to the interactions between the multimerizing components (the third fusion polypeptide components). Producing the fusion polypeptides in this manner avoids the need for purification of heterodimeric mixtures that would result if the first and second components were produced as separate molecules and then multimerized. For example, U.S. Patent No. 5,470,952 issued November 28, 1995 describes the production of heterodimeric proteins that function as CNTF or IL-6 antagonists. The heterodimers are purified from cell lines cotransfected with the appropriate alpha ( $\alpha$ ) and beta ( $\beta$ ) components. Heterodimers are then separated from homodimers using methods such as passive elution from preparative, nondenaturing polyacrylamide gels or by using high pressure 20 cation exchange chromatography. The need for this purification step is avoided by the methods of the present invention.

In addition, PCT International Application WO 96/11213 published 18 April 1996 entitled Dimeric IL-4 Inhibitors states that the applicant has prepared homodimers in which two IL-4 receptors are bound by a polymeric spacer and has prepared heterodimers in which an IL-4 receptor is linked by a polymeric spacer to an IL-2 receptor gamma chain. The polymeric spacer described is polyethylene glycol (PEG). The two receptor components, IL-4R and IL-2Rgamma are separately expressed and purified. Pegylated homodimers and heterodimers are then produced by joining the components together using bi-functional PEG reagents. It is an advantage

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of the present invention that it avoids the need for such time consuming and costly purification and pegylation steps.

In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the invention may be prepared in which the order of the first, second and third fusion polypeptide components are rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, leukemia inhibitory factor, and cardiotrophin-1.

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In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

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In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the immunoglobulin superfamily

of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

In still further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18, and MIF.

Because specificity determination and signal transduction occurs by a

similar mechanism in the TGF-β/BMP family of cytokines (See D.

Kingsley, Genes & Development, 1994, 8: 133-146; J. Wrana, Miner
Electrolyte Metab, 24: 120-130 (1998); R. Derynck and X. Feng, Biochimica et
Biophysica Acta 1333 (1997) F105-F150; and J. Massague and F. Weis-Garcia,

"Serine/threonine Kinase Receptors: Mediators of Transforming Growth

Factor Beta Family Signals" In Cancer Surveys, Vol. 27: Cell Signaling,

1996, Imperial Cancer Research Fund) the present invention may be used
to produce high affinity antagonists for cytokines that are members of the

TGF-β/BMP family.

Therefore, in additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TGF-β/BMP family selected from the group consisting of TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian

inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

In alternative embodiments of the invention, the specificity determining component, the signal transducing component, or both, may be substituted 5 for by a single chain Fv. A single chain Fv (scFv) is a truncated Fab having only the V region of a heavy chain linked by a stretch of synthetic peptide to a V region of a light chain. See, for example, US Patent Nos. 5,565,332; 5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge Antibody Technology Limited incorporated by reference herein. Thus the 10 present invention contemplates, for example, an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the 15 specificity determining component of the cytokine's receptor; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of an scFv capable of binding the cytokine at a site different from the site at which the cytokine binding portion of the 20 extracellular domain of the specificity determining component of the cytokine's receptor binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component. Alternatively, the specificity determining component may be substituted for by a scFv that binds to a site on the 25 cytokine different from the site at which the signal transducing component binds. Thus the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid 30 sequence of a scFv that binds to a site on the cytokine different from the site at which the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor binds; a nucleotide sequence encoding a second fusion polypeptide component

comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In another embodiment, the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a first scFv that binds to a site on the cytokine; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence a second scFv that binds to a site on the cytokine different from the site at which the first scFv binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In all of the above described embodiments comprising scFv's, the invention also contemplates embodiments in which the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component; embodiments in which the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component; and further embodiments of the invention in which the order of the first, second and third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

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In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc( $\Delta$ C1). Alternatively, the multimerizing component may be an Fc domain in which a cysteine within the first five amino acids has been substituted for by another amino acid such as, for example, serine or alanine.

The present invention also provides for fusion polypeptides encoded by the isolated nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the third multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, Cell 29:671-679); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the third multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion

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polypeptide. The suitable host cell may be a bacterial cell such as <u>E. coli</u>, a yeast cell, such as <u>Pichia pastoris</u>, an insect cell, such as <u>Spodoptera</u> frugiperda, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

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The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

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The present invention provides novel antagonists which are based on receptor components that are shared by cytokines such as the CNTF family of cytokines.

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The invention described herein contemplates the production of antagonists to any cytokine that utilizes an  $\alpha$  specificity determining component which, when combined with the cytokine, binds to a first  $\beta$ signal transducing component to form a nonfunctional intermediate which then binds to a second  $\beta$  signal transducing component causing  $\beta$ receptor dimerization and consequent signal transduction. According to the invention, the soluble  $\alpha$  specificity determining component of the receptor (sR $\alpha$ ) and the extracellular domain of the first  $\beta$  signal transducing component of the cytokine receptor (β1) are combined to form heterodimers ( $sR\alpha$ : $\beta$ 1) that act as antagonists to the cytokine by binding the cytokine to form a nonfunctional complex.

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As described in Example 1, CNTF and IL-6 share the  $\beta$ 1 receptor component gp130. The fact that CNTF forms an intermediate with CNTFR $\alpha$  and gp130 can be demonstrated (Example 1) in cells lacking LIFRβ, where the complex of CNTF and CNTFRα binds gp130, and

prevents homodimerization of gp130 by IL-6 and IL-6R $\alpha$ , thereby blocking signal transduction. These studies provide the basis for the development of the IL-6 antagonists described herein, as they show that if, in the presence of a ligand, a nonfunctional intermediate complex, consisting of the ligand, its  $\alpha$  receptor component and its  $\beta$ 1 receptor component, can be formed, it will effectively block the action of the ligand. Other cytokines may use other  $\beta$ 1 receptor components, such as LIFR $\beta$ , which may also be used to produce antagonists according to the present invention.

- Thus for example, in one embodiment of the invention, effective antagonists of IL-6 or CNTF consist of heterodimers of the extracellular domains of the α specificity determining components of their receptors (sIL-6Rα and sCNTFRα, respectively) and the extracellular domain of gp130. The resultant heterodimers, which are referred to hereinafter as sIL-6Rα:β1 and sCNTFRα:β1, respectively, function as high-affinity traps for IL-6 or CNTF, respectively, thus rendering the cytokine inaccessible to form a signal transducing complex with the native membrane-bound forms of their receptors.
- Although soluble ligand binding domains from the extracellular portion of receptors have proven to be somewhat effective as traps for their ligands and thus act as antagonists [Bargetzi, et al., Cancer Res. 53:4010-4013 (1993); , et al., Proc. Natl. Acad. Sci. USA 89: 8616-8620 (1992); Mohler, et al., J. Immunol. 151: 1548-1561 (1993); Narazaki, et al., Blood 82: 1120-1126 (1993)],
- the IL-6 and CNTF receptors are unusual in that the α receptor components constitute ligand binding domains that, in concert with their ligands, function effectively in soluble form as receptor agonists [Davis, et al. Science 259:1736-1739 (1993); Taga, et al., Cell 58: 573-581 (1989)]. The sRα:β1 heterodimers prepared according to the present invention provide effective traps for their ligands, binding these ligands with affinities in the picomolar range (based on binding studies for CNTF to PC12D cells)

without creating functional intermediates. The technology described herein may be applied to develop a cytokine trap for any cytokine that utilizes an  $\alpha$ -component that confers specificity, as well as a  $\beta$  component which, when bound to the  $\alpha$ -specificity component, has a higher affinity for the cytokine than either component alone. Accordingly, antagonists according to the invention include antagonists of interleukins 1 through 5 [IL-1, Greenfeder, et al. J. Biol. Chem. 270:13757-13765 (1995); Guo, et al. J. Biol. Chem. 270:27562-27568 (1995)], IL-2; [Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)]; IL-3; [Kitamura, et al. Cell 66:1165-1174 (1991)], IL-4; [Idzerda, et al. J. Exp. Med. 171:861-873 (1990)], IL-5; [Taverneir, et al. Cell 66:1175-1184 (1991)], IL-11 [(Cherel, et al. Direct Submission to EMBL/GenBank/DDB] databases; accession No. Z38102)], interleukin 15 [IL-15; Hemar, et al. J. Cell Biol. 1295:55-64 (1995); Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)], granulocyte-macrophage colony stimulating factor [GM-CSF; Hayashida, et al. Proc. Natl. Acad. Sci. U.S.A. 97:9655-9659 (1990)], LIF, gamma interferon [IFNy; Aguet, et al. Cell 55:273-280 (1988); Soh, et al. Cell 76:793-802 (1994)], and transforming growth factor beta [TGFβ; Inagaki, et al. Proc. Natl. Acad. Sci. USA 90:5359-5363 (1993)].

The  $\alpha$  and  $\beta$  receptor extracellular domains may be prepared using methods known to those skilled in the art. The CNTFR $\alpha$  receptor has been cloned, sequenced and expressed [Davis, et al. (1991) Science 253:59-63 which is incorporated by reference in its entirety herein]. The cloning of LIFR $\beta$  and gp130 are described in Gearing et al. in EMBO J. 10:2839-2848 (1991), Hibi, et al. Cell 63:1149-1157 (1990) and in published PCT application WO 93/10151 published May 27, 1993, all of which are incorporated by reference in their entirety herein.

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The receptor molecules useful for practicing the present invention may be prepared by cloning and expression in a prokaryotic or eukaryotic expression system. The recombinant receptor gene may be expressed and purified utilizing any number of methods. The gene encoding the factor may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The recombinant factors may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The sRα:β heterodimeric receptors may be engineered using known fusion regions, as described in published PCT application WO 93/10151 published May 27, 1993 entitled "Receptor for Oncostatin M and Leukemia Inhibitory Factor" which describes production of β receptor heterodimers, or they may be prepared by crosslinking of extracellular domains by chemical means. The domains utilized may consist of the entire extracellular domain of the α and β components, or they may consist of mutants or fragments thereof that maintain the ability to form a complex with its ligand and other components in the sRα:β1 complex. For example, as described below in Example 4, IL-6 antagonists have been prepared using gp130 that is lacking its three fibronectin-like domains.

In one embodiment of the invention, the extracellular domains are engineered using leucine zippers. The leucine zipper domains of the human transcription factors c-jun and c-fos have been shown to form stable heterodimers [Busch and Sassone-Corsi, Trends Genetics 6: 36-40]

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(1990); Gentz, et al., Science 243: 1695-1699 (1989)] with a 1:1 stoichiometry. Although jun-jun homodimers have also been shown to form, they are about 1000-fold less stable than jun-fos heterodimers. Fos-fos homodimers have not been detected.

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The leucine zipper domain of either c-jun or c-fos are fused in frame at the C-terminus of the soluble or extracellular domains of the above mentioned receptor components by genetically engineering chimeric genes. The fusions may be direct or they may employ a flexible linker domain, such as the hinge region of human IgG, or polypeptide linkers consisting of small amino acids such as glycine, serine, threonine or alanine, at various lengths and combinations. Additionally, the chimeric proteins may be tagged by His-His-His-His-His-His (His6),[SEQ. ID NO. 1] to allow rapid purification by metal-chelate chromatography, and/or by epitopes to which antibodies are available, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

In another embodiment, as described below in Example 3, the sRα:β1 20 heterodimer is prepared using a similar method, but using the Fc-domain of human IgG1 [Aruffo, et al., Cell 67:35-44 (1991)]. In contrast to the latter, formation of heterodimers must be biochemically achieved, as chimeric molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers. Thus, homodimers may be reduced under conditions that 25 favor the disruption of inter-chain disulfides but do not effect intra-chain disulfides. Then monomers with different extracellular portions are mixed in equimolar amounts and oxidized to form a mixture of homoand heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of 30 heterodimers may be biased by genetically engineering and expressing molecules that consist of the soluble or extracellular portion of the receptor components followed by the Fc-domain of hIgG, followed by

either the c-jun or the c-fos leucine zippers described above [Kostelny, et al., J. Immunol. 148: 1547-1553 (1992)]. Since these leucine zippers form predominately heterodimers, they may be used to drive formation of the heterodimers where desired. As for the chimeric proteins described using leucine zippers, these may also be tagged with metal chelates or an epitope. This tagged domain can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

- In additional embodiments, heterodimers may be prepared using other 10 immunoglobulin derived domains that drive the formation of dimers. Such domains include, for example, the heavy chains of IgG (Cy1 and Cy4), as well as the constant regions of kappa ( $\kappa$ ) and lambda ( $\lambda$ ) light chains of human immunoglobulins. The heterodimerization of Cy with the light chain occurs between the CH1 domain of Cy and the constant region of the 15 light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. Accordingly, as described in Example 4, constructs may be prepared using these immunoglobulin domains. Alternatively, the immunoglobulin domains include domains that may 20 be derived from T cell receptor components which drive dimerization. In another embodiment of the invention, the  $sR\alpha$ : $\beta$ 1 heterodimers are prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two soluble or extracellular domains fused together in tandem ("head to head") by a flexible loop. This loop may be entirely 25 artificial (e.g. polyglycine repeats interrupted by serine or threonine at a certain interval) or "borrowed" from naturally occurring proteins (e.g. the hinge region of hIgG). Molecules may be engineered in which the order of the soluble or extracellular domains fused is switched (e.g.
- $30 sIL6R\alpha/loop/sgp130 or sgp130/loop/sIL-6R\alpha)$  and/or in which the length

and composition of the loop is varied, to allow for selection of molecules with desired characteristics.

Alternatively, the heterodimers made according to the present invention

5 may be purified from cell lines cotransfected with the appropriate α and β
components. Heterodimers may be separated from homodimers using
methods available to those skilled in the art. For example, limited
quantities of heterodimers may be recovered by passive elution from
preparative, nondenaturing polyacrylamide gels. Alternatively,

10 heterodimers may be purified using high pressure cation exchange
chromatography. Excellent purification has been obtained using a Mono S
cation exchange column.

In addition to sRα:β1 heterodimers that act as antagonists by binding free CNTF or IL-6, the present invention also contemplates the use of engineered, mutated versions of IL-6 with novel properties that allow it to bind to IL-6Rα and a single gp130 molecule, but fail to engage the second gp130 to complete  $\beta$  component homodimerization, and thus act as an effective IL-6 antagonist on any IL-6 responsive cell. Our model for the structure of the IL-6 and CNTF receptor complexes indicates that these cytokines have distinct sites for binding the  $\alpha$ ,  $\beta$ 1, and  $\beta$ 2 receptor components [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Mutations of critical amino acid residues comprising each of these sites gives rise to novel molecules which have the desired antagonistic properties. Ablation of the  $\beta$ 1 site would give a molecule which could still bind to the  $\alpha$ receptor component but not the β1 component, and thereby comprise an antagonist with nanomolar affinity. Mutations of critical amino acid residues comprising the  $\beta2$  site of IL-6 (IL-6 $\beta2$ -) would give a molecule that would bind to IL-6Rα and the first gp130 monomer, but fail to engage the second gp130 and thus be functionally inactive. Similarly, mutations of

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the CNTF  $\beta 2$  site would give a molecule (CNTF $\beta 2$ -) that would bind CNTFR $\alpha$  and gp130, but fail to engage LIFR $\beta$ , thereby antagonizing CNTF action by forming the non-functional  $\beta 1$  intermediate. Based on the binding results described above where CNTF forms the  $\beta 1$  intermediate with high affinity, both CNTF $\beta 2$ - and IL-6 $\beta 2$ - would constitute antagonists with affinity in the range of 10 pM.

A variety of means are used to generate and identify mutations of IL-6 or CNTF that have the desired properties. Random mutagenesis by standard methods of the DNA encoding IL-6 or CNTF may be used, followed by analysis of the collection of products to identify mutated cytokines having the desired novel properties as outlined below. Mutagenesis by genetic engineering has been used extensively in order to elucidate the structural organization of functional domains of recombinant proteins. Several different approaches have been described in the literature for carrying out deletion or substitution mutagenesis. The most successful appear to be alanine scanning mutagenesis [Cunningham and Wells (1989), Science 244: 1081-1085] and homolog-scanning mutagenesis [Cunningham, et al., (1989), Science 243:1330-1336].

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Targeted mutagenesis of the IL-6 or CNTF nucleic acid sequences using such methods can be used to generate CNTF $\beta$ 2- or IL-6 $\beta$ 2- candidates. The choice of regions appropriate for targeted mutagenesis is done systematically, or determined from studies whereby panels of monoclonal antibodies against each factor are used to map regions of the cytokine that might be exposed after binding of the cytokine to the  $\alpha$  receptor component alone, or to the  $\alpha\beta1$  heterodimeric soluble receptors described above. Similarly, chemical modification or limited proteolysis of the cytokine alone or in a complex bound to the  $\alpha$  receptor component or the  $\alpha\beta1$  heterodimeric soluble receptors described above, followed by analysis

of the protected and exposed regions could reveal potential  $\beta$ 2 binding sites.

Assays for identifying CNTF or IL-6 mutants with the desired properties involve the ability to block with high affinity the action of IL-6 or CNTF on appropriately responsive cell lines [Davis, et al., Science 259: 1736-1739 (1993); Murakami, et al., Proc. Natl. Acad. Sci. USA 88: 11349-11353 (1991)]. Such assays include cell proliferation, survival, or DNA synthesis driven by CNTF or IL-6, or the construction of cell lines where binding of factor induces production of reporters such as CAT or  $\beta$ -galactosidase [Savino, et al., Proc. Natl. Acad. Sci. USA 90: 4067-4071 (1993)].

Alternatively, the properties of various mutants may be assessed with a receptor-based assay. One such assay consists of screening mutants for their ability to bind the  $sR\alpha:\beta1$  receptor heterodimers described above using epitope-tagged [Davis et al., Science 253: 59-63 (1991)]  $sR\alpha:\beta1$  reagents. Furthermore, one can probe for the presence or absence of the  $\beta2$  site by assessing whether an epitope-tagged soluble  $\beta2$  reagent will bind to the cytokine in the presence of the  $\beta1$  heterodimer. For example, CNTF only binds to LIFR $\beta$  (the  $\beta2$  component) in the presence of both CNTFR $\alpha$  and gp130 [Davis, et al. Science 260: 1805-1808 (1993); Stahl, et al. J. Biol. Chem. 268: 7628-7631 (1993)]. Thus a soluble LIFR $\beta$  reagent would only bind to CNTF in the presence of the soluble  $sR\alpha:\beta1$  dimer  $sCNTFR\alpha:\beta1$ . For IL-6, the  $sR\alpha:\beta1$  reagent would be IL-6 $R\alpha:\beta1$ , and the probe for the  $\beta2$  site would be epitope-tagged sgp130. Thus  $\beta2$ - mutants of CNTF would be identified as those that bound the  $sR\alpha:\beta1$  reagent, demonstrating that the  $\alpha$  and  $\beta1$  site of the cytokine were intact, yet failed to bind the  $\beta2$  reagent.

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In addition, the present invention provides for methods of detecting or measuring the activity of potential  $\beta$ 2- mutants by measuring the phosphorylation of a  $\beta$ -receptor component or a signal transduction component selected from the group consisting of Jak1, Jak2 and Tyk2 or any other signal transduction component, such as the CLIPs, that are determined to be phosphorylated in response to a member of the CNTF family of cytokines.

A cell that expresses the signal transduction component(s) described

herein may either do so naturally or be genetically engineered to do so.

For example, Jak1 and Tyk-2-encoding nucleic acid sequences obtained as described in Velazquez, et al., Cell, Vol. 70:313-322 (1992), may be introduced into a cell by transduction, transfection, microinjection, electroporation, via a transgenic animal, etc., using any known method known in the art.

According to the invention, cells are exposed to a potential antagonist and the tyrosine phosphorylation of either the  $\beta$ -component(s) or the signal transduction component(s) are compared to the tyrosine phosphorylation of the same component(s) in the absence of the potential antagonist. In another embodiment of the invention, the tyrosine phosphorylation that results from contacting the above cells with the potential antagonist is compared to the tyrosine phosphorylation of the same cells exposed to the parental CNTF family member. In such assays, the cell must either express the extracellular receptor ( $\alpha$ -component) or the cells may be exposed to the test agent in the presence of the soluble receptor component. Thus, for example, in an assay system designed to identify agonists or antagonists of CNTF, the cell may express the  $\alpha$ - component CNTFR $\alpha$ , the  $\beta$ -components gp130 and LIFR $\beta$  and a signal transducing component such as Jak1. The cell is exposed to test agents, and the tyrosine phosphorylation of either the  $\beta$ -components or the signal transducing component is

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compared to the phosphorylation pattern produced in the presence of CNTF. Alternatively, the tyrosine phosphorylation which results from exposure to a test agent is compared to the phosphorylation which occurs in the absence of the test agent. Alternatively, an assay system, for example, for IL-6 may involve exposing a cell that expresses the  $\beta$ -component gp130 and a signal transducing protein such as Jak1, Jak2 or Tyk2 to a test agent in conjunction with the soluble IL-6 receptor.

In another embodiment of the invention the above approaches are used to develop a method for screening for small molecule antagonists that act at 10 various steps in the process of ligand binding, receptor complex formation, and subsequent signal transduction. Molecules that potentially interfere with ligand-receptor interactions are screened by assessing interference of complex formation between the soluble receptors and ligand as described 15 above. Alternatively, cell-based assays in which IL-6 or CNTF induce response of a reporter gene are screened against libraries of small molecules or natural products to identify potential antagonists. Those molecules showing antagonist activity are rescreened on cell-based assays responding to other factors (such as GM-CSF or factors like Neurotrophin-20 3 that activate receptor tyrosine kinases) to evaluate their specificity against the CNTF/IL-6/OSM/LIF family of factors. Such cell-based screens are used to identify antagonists that inhibit any of numerous targets in the signal transduction process.

In one such assay system, the specific target for antagonists is the interaction of the Jak/Tyk family of kinases [Firmbach-Kraft, Oncogene 5: 1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11:2057-2065 (1991)] with the receptor β subunits. As described above, LIFRβ and gp130 preassociate with members of the Jak/Tyk family of cytoplasmic protein tyrosine kinases, which become activated in response to ligand-induced β component dimerization Stahl, et al. Science 263:92-95 (1993). Thus small molecules that could enter the cell cytoplasm and disrupt the interaction

between the  $\beta$  component and the Jak/Tyk kinase could potentially block all subsequent intracellular signaling. Such activity could be screened with an in vitro scheme that assessed the ability of small molecules to block the interaction between the relevant binding domains of purified \$\beta\$ component and Jak/Tyk kinase. Alternatively, one could easily screen for molecules that could inhibit a yeast-based assay of  $\beta$  component binding to Jak/Tyk kinases using the two-hybrid interaction system [Chien, et al., Proc. Natl. Acad. Sci. 88: 9578-9582 (1991)]. In such a system, the interaction between two proteins ( $\beta$  component and Jak/Tyk kinase or relevant domains thereof in this example) induces production of a convenient marker such as  $\beta$ - galactosidase. Collections of small molecules are tested for their ability to disrupt the desired interaction without inhibiting the interaction between two control proteins. The advantage of this screen would be the requirement that the test compounds enter the cell before inhibiting the interaction between the β component and the Jak/Tyk kinase.

The CNTF family antagonists described herein either bind to, or compete with the cytokines CNTF and IL-6. Accordingly, they are useful for treating diseases or disorders mediated by CNTF or IL-6. For example, therapeutic uses of IL-6 antagonists would include the following:

1) In osteoporosis, which can be exacerbated by lowering of estrogen levels in post-menopausal women or through ovariectomy, IL-6 appears to be a critical mediator of osteoclastogenesis, leading to bone resorption [Horowitz, Science 260: 626-627 (1993); Jilka, et al., Science 257: 88-91 (1992)]. Importantly, IL-6 only appears to play a major role in the estrogen-depleted state, and apparently is minimally involved in normal bone maintenance. Consistent with this, experimental evidence indicates that function-blocking antibodies to IL-6 can reduce the number of osteoclasts [Jilka, et al. Science 257: 88-91 (1992)]. While estrogen replacement therapy is also used, there appear to be side effects that may include increased risk of

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endometrial and breast cancer. Thus, IL-6 antagonists as described herein would be more specific to reduce osteoclastogenesis to normal levels.

- 2) IL-6 appears to be directly involved in multiple myeloma by acting in either an autocrine or paracrine fashion to promote tumor formation [van Oers, et al., Ann Hematol. 66: 219-223 (1993)]. Furthermore, the elevated IL-6 levels create undesirable secondary effects such as bone resorption, hypercalcemia, and cachexia; in limited studies function-blocking antibodies to IL-6 or IL-6Ra have some efficacy [Klein, et al., Blood 78: 1198-1204 (1991); Suzuki, et al., Eur. J. Immunol. 22:1989-1993 (1992)]. Therefore, IL-6 antagonists as described herein would be beneficial for both the secondary effects as well as for inhibiting tumor growth.
- 3) IL-6 may be a mediator of tumor necrosis factor (TNF) that leads to cachexia associated with AIDS and cancer [Strassmann, et al., J. Clin. Invest. 89: 1681-1684 (1992)], perhaps by reducing lipoprotein lipase activity in adipose tissue [Greenberg, et al., Cancer Research 52: 4113-4116 (1992)]. Accordingly, antagonists described herein would be useful in alleviating or reducing cachexia in such patients.
- Effective doses useful for treating these or other CNTF family related diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975)]. Pharmaceutical compositions for use according to the invention include the antagonists described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation (including antagonist expressing cells) prior to administration in vivo. For example, the pharmaceutical composition may comprise one or more of the antagonists in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such

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treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

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Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

### **EXAMPLES**

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#### EXAMPLE 1: CNTF COMPETES WITH IL-6 FOR BINDING TO GP130

#### MATERIALS AND METHODS

- Materials. A clone of PC12 cells that respond to IL-6 (PC12D) was obtained from DNAX. Rat CNTF was prepared as described [Masiakowski, et al., J. Neurochem. 57:1003-10012 (1991)]. IL-6 and sIL-6Rα were purchased from R & D Systems. Antisera was raised in rabbits against a peptide derived from a region near the C-terminus of gp130 (sequence:
- 25 CGTEGQVERFETVGME) [SEQ. ID. NO. 2] by the method described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993). Anti-phosphotyrosine monoclonal 4G10 was purchased from UBI, and reagents for ECL from Amersham.
- 30 <u>Signal Transduction Assays</u>. Plates (10 cm) of PC12D were starved in serum-free medium (RPMI 1640 + glutamine) for 1 hour, then incubated with IL-6 (50 ng/mL) + sIL-6R (1 mg/mL) in the presence or absence of

added rat CNTF at the indicated concentrations for 5 minutes at 37°C. Samples were then subjected to anti-gp130 immunoprecipitation, SDS PAGE, and anti-phosphotyrosine immunoblotting as described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993).

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#### **RESULTS**

The ability of CNTF to block IL-6 responses was measured using a PC12 cell line (called PC12D) that expresses IL-6R $\alpha$ , gp130, and CNTFR $\alpha$ , but not LIFR $\beta$ . As one would predict, these cells respond to IL-6, but not to CNTF 10 (Fig. 2) since LIFR $\beta$  is a required component for CNTF signal transduction [Davis, et al., Science 260: 59-63 (1993)]. In accordance with results on other cell lines [Ip, et al., Cell 69: 1121-1132 (1992)], PC12D cells give tyrosine phosphorylation of gp130 (as well as a variety of other proteins called CLIPs) in response to 2 nM IL-6 (Fig. 2). Addition of recombinant soluble 15 IL-6R $\alpha$  (sIL-6R $\alpha$ ) enhances the level of gp130 tyrosine phosphorylation, as has been reported in some other systems [(Taga, et al., Cell 58: 573-581 (1989)]. However, addition of 2 nM CNTF simultaneously with IL-6 severely diminishes the tyrosine phosphorylation of gp130. Although a slight gp130 phosphorylation response remains in the presence of CNTF, 20 IL-6, and sIL-6R $\alpha$ , it is eliminated if the CNTF concentration is increased fourfold to 8 nM. Thus, in IL-6 responsive cells that contain CNTFR $\alpha$  but no LIFR $\beta$ , CNTF is a rather potent antagonist of IL-6 action.

# 25 EXAMPLE 2. BINDING OF CNTF TO THE CNTFRα:β

#### MATERIALS AND METHODS

Scatchard Analysis of CNTF Binding. 125I-CNTF was prepared and purified as described [Stahl et al. JBC 268: 7628-7631 (1993)]. Saturation binding studies were carried out in PC12 cells, using concentrations of 125I-

CNTF ranging from 20pM to 10nM. Binding was performed directly on a monolayer of cells. Medium was removed from wells and cells were washed once with assay buffer consisting of phosphate buffered saline (PBS; pH 7.4), 0.1mM bacitracin, 1mM PMSF, 1mg/ml leupeptin, and 5 1mg/ml BSA. Cells were incubated in 125I-CNTF for 2 hours at room temperature, followed by 2 quick washes with assay buffer. Cells were lysed with PBS containing 1% SDS and counted in a Packard Gamma Counter at 90-95% efficiency. Non-specific binding was defined by the presence of 100-fold excess of unlabelled CNTF. Specific binding ranged 10 from 70% to 95%.

#### **RESULTS**

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The equilibrium constant for binding of CNTF to CNTFRa:\(\beta\)1 was estimated from Scatchard analysis of iodinated CNTF binding on PC12D cells (Figure 3). The data is consistent with a 2 site fit having dissociation constants of 9 pM and 3.4 nM. The low affinity site corresponds to interaction of CNTF with CNTFRa, which has a Kd near 3 nM [(Panayotatos, et al., J. Biol. Chem. 268: 19000-19003 (1993)]. We interpret the high affinity complex as the intermediate containing CNTF, CNTFR $\alpha$ , and gp130. A Ewing sarcoma cell line (EW-1) which does contain CNTFR $\alpha$ , gp130, and LIFR $\beta$ , and therefore gives robust tyrosine phosphorylation in response to CNTF, displays a very similar two site fit with dissociation constants of 1 nM and 10. Thus it is apparent that CNTF 25 binds with equally high affinity to a complex containing only CNTFRa and gp130, as it does to a complex which additionally contains LIFRβ, thus demonstrating the feasibility of creating the sRα:β antagonists described herein.

#### EXAMPLE 3. METHODS OF PRODUCING CYTOKINE LIGAND TRAPS

#### Virus Stock Production

5 SF21 insect cells obtained from *Spodoptera frugiperda* were grown at 27°C in Gibco SF900 II medium to a density of 1x10⁶ cells/mL. The individual virus stock for either GP130-Fc-His6 (Figure 4) or IL6Ra-Fc (Figure 5) was added to the bioreactor to a low multiplicity 0.01-0.1 PFU/cell to begin the infection. The infection process was allowed to continue for 5-7 days allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically aliquoted into sterile centrifuge bottles and the cells removed by centrifugation. The cell-free supernatant was collected in sterile bottles and stored at 4°C until further use.

The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 2x10⁶ cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow the virus to adsorb to individual cells. An agar overlay is added and plates incubated for 5 - 7 days at 27°C. Staining of viable cells with neutral red revealed circular plaques resulting which were counted to give the virus titer.

#### Coinfection of Cells for Protein Production

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Uninfected SF21 Cells were grown in a 60L ABEC bioreactor containing 40L of SF900 II medium. Temperature was controlled at 27°C and the dissolved oxygen level was maintained at 50% of saturation by controlling the flowrate of oxygen in the inlet gas stream. When a density of 2x106 cells/mL was reached, the cells were concentrated within the bioreactor to a volume of 20L using a low shear steam sterilizable pump with a tangential flow filtration device with Millipore Prostak 0.65 micron

membranes. After concentration fresh sterile growth medium is slowly added to the bioreactor while the filtration system continues to remove the spent growth medium by diafiltration. After two volume exchanges (40L) have been carried out an additional 20L of fresh medium was added to the bioreactor to resuspend the cells to the original volume of 40L. The cell density was determined once again by counting viable cells using a hemacytometer.

The required amount of each virus stock was calculated based on the cell
density, virus titer and the desired multiplicity of infection (MOI). Virus
stock ratios of 5:1, 5:2, 10:2 and 10:4, IL6Rα-Fc to GP130-Fc-His6 all resulted
in production of significant amounts of heterodimer. The ideal virus
stock ratio is highly dependent on the ease of purification of the
heterodimer from each of the two homodimers. The IL6Rα-Fc

15 homodimer is relatively easy to remove downstream by immobilized
metal affinity chromatography. Virus infection ratios have been chosen to
minimize the formation of the GP130-Fc-His6 homodimer which is more
difficult to clear downstream. The relative amount of GP130-Fc-His6 virus
stock chosen for infection has increased with successive batches as the

20 purification method for clearing the resultant homodimer has improved.

The virus stocks were aseptically mixed in a single vessel then transferred to the bioreactor. This results in synchronous infection of the SF21 cells. The infection is allowed to proceed for three to four days, allowing sufficient time for maximal production of the heterodimer protein.

# Recovery and Protein A Chromatographic Purification

At the conclusion of the infection phase of the bioreactor process the cells were concentrated in the bioreactor using a 10 ft² Millipore Prostak filter (0.65 micron) pore size. The cell-free permeate passing through the filter was collected in a clean process vessel. At the conclusion of the filtration

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operation the pH of permeate stream, containing the protein product, was adjusted to 8.0 with 10N NaOH. The resultant precipitate was removed by forcing the extract through a 0.8 micron depth filter (Sartorious), followed by a 0.2 micron filter. Sufficient 0.5M EDTA stock was added to give a final concentration of 5mM. The filtered protein solution was loaded onto a 10 cm diameter column containing 100-200 mL of Pharmacia Protein A Sepharose 4 Fast Flow, equilibrated with PBS. Protein A has a very high affinity for the Fc-Fc domain of each of the 3 recombinant protein products, allowing them to bind while other proteins in the cell-free extract flow through the column. After loading the column was washed to baseline with PBS containing an additional 350mM NaCl. The IgG-Fc tagged proteins were eluted at low pH, either with 0.5M acetic acid or with a decreasing pH gradient of 0.1M citric acid and 0.2M disodium phosphate buffers. Tris base or disodium phosphate was added to the eluted protein to avoid prolonged exposure to low pH conditions.

The pooled protein was diafiltered into PBS or HEPES buffer and derivitized with 1 mM iodoacetamide to protect the exposed sulfhydryl group on the free cysteine near the hinge region of each Fc domain. This prevents disulfide mediated aggregation of proteins. A 6 ft² Millipore spiral wound ultrafiltration membrane with nominal 30 kiloDalton cutoff was used to perform the buffer exchange. The total protein was determined by UV absorbance at 280 nm using the diafiltration buffer as a blank. The relative amounts of heterodimer and two homodimer proteins were determined by SDS PAGE gel electrophoresis using a 6% Tris-Glycine gel (Novex). Gels were Coomassie-stained then transferred into destain solution overnight. A Shimadzu scanning densitometer was used to determine the relative intensity of the individual protein bands on the SDS PAGE gel. The peak area ratios are used to compute the fraction of heterodimer and each of the homodimers in the column pool fractions.

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# Immobilized Metal Affinity Chromatographic Purification

The six histidine residues on the C-terminus of the GP130-Fc-His6 fusion protein provides an excellent molecular handle for separation of the heterodimeric IL6 antagonist from the two homodimers. The imidazole group on each of the C-terminal histidines of the GP130-Fc-His6 moiety has a strong binding constant with several divalent metals, including copper, nickel, zinc, cobalt, iron and calcium. Since the IL6Rα-Fc homodimer has no C-terminal histidine residues, it clearly has the lowest affinity. The IL6Rα-Fc-GP130-Fc-His6 heterodimer has a single stand set six histidines giving it greater affinity for the metal, while the GP130-Fc-His6 homodimer has two sets of six histidines each giving it the highest affinity of the three IgG tagged proteins to the metal affinity column. Selective elution of the three proteins with increasing amounts of imidazole in the elution buffer therefore elutes the proteins in the following order:

- 1. IL6Rα-Fc homodimer
- 2. IL6Rα-Fc-GP130-Fc-His heterodimer
- 20 3. GP130-Fc-His homodimer

A 26 mm diameter column containing 100 mL of Pharmacia Chelating. Sepharose Fast Flow was saturated with a solution of nickel sulfate until a significant green color is observed in the column eluate. The column is then washed with several column volumes of deionized water, then equilibrated with 50 mM HEPES, 40mM imidazole, pH 8.0. The binding of imidazole to the immobilized nickel results in a green to blue color change. Imidazole was added to the protein load to a final concentration of 40mM. Addition of imidazole to the protein load reduces the binding of IL6Rα-Fc homodimer, increasing the surface area available for the remaining two species. After loading, the column was washed with

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several column volumes of 50 mM HEPES, 80mM imidazole, pH 8.0 until a steady baseline was reestablished. The heterodimer was selectively eluted with 50 mM HEPES, 150mM imidazole, pH 8.0 over several column volumes. The protein fractions were pooled and diafiltered into PBS as described in the section above.

# EXAMPLE 4. ALTERNATIVE METHODS OF CONSTRUCTING LIGAND TRAPS

10 As described above, receptor activation by CNTF, and analogously by IL-6 and IL-11, follows an ordered sequence of binding events (Figure 6). The cytokine initially binds to its cognate R $\alpha$  with low affinity (Kd = 3 to 10 nM); this is a required step - cells which do not express the cognate Rα do not respond to the cognate cytokine. The cytokine•Rα complex associates 15 with the first signal transducing component, gp130, to form a high affinity complex (Kd in the order of 10 pM for the CNTF•CNTFRα•gp130 complex). This complex does not transduce signal, as it is the dimerization of the signal transducing components that brings about signaling (Stahl and Yancopoulos, J. Neurobiology 25: 1454-1466 (1994); Stahl et al., Science 20 267:1349-1353 (1995); Davis et al., Science 260:1805-1808 (1993); Stahl et al., Science 263:92-95 (1994); Murakami, et al. Science 260:1808-1810 (1993). At least in the case of IL-6, the cytokine•Rα•signal transducer heterotrimeric complex subsequently associates with another like complex, to form a hexameric complex (Figure 6) (Ward et al., J. Biol. Chem. 269:23286-23289 25 (1994). The resulting dimerization of the signal transducers - gp130 in the case of IL-6 (Murakami et al., Science 260:1808-1810 (1993) and IL-11, gp130 and LIFR in the case of CNTF (Davis et al., Science 260:1805-1808 (1993) brings about signal transduction.

30 The initial heterodimeric molecules made comprised a soluble Rαcomponent linked to the extracellular domain of gp130. These molecules

were shown to mimic the high affinity cytokine  $R\alpha \cdot gp130$  complex and behave as a high affinity antagonist of their cognate cytokine (Figure 7). To make these molecules, the extracellular domain of gp130 was paired with the extracellular domain of the  $\alpha$ -receptor components for IL-6 and CNTF,

IL-6Rα and CNTFRα respectively. To link the Rα with the extracellular domain of gp130, the soluble Rα-components and gp130 were fused to the Fc portion of human IgG1 to produce Rα-Fc and gp130-Fc respectively. The Fc domain was chosen primarily but not solely because it naturally forms disulfide-linked dimers. Heterodimeric molecules comprising Rα-Fc•gp130-Fc were expressed, purified and shown to behave as highly potent antagonists of their cognate ligand. Furthermore, these molecules were found to be highly specific for their cognate cytokine since it is the choice of the α-receptor component which specifies which cytokine is bound and trapped (there is no measurable binding of the cytokine to

Here we describe an extension of this technology which allows the engineering of different heteromeric soluble receptor ligand traps which by virtue of their design may have additional beneficial characteristics such as stability, Fc-receptor-mediated clearance, or reduced effector functions (such as complement fixation). Furthermore, the technology described should prove suitable for the engineering of any heteromeric protein in mammalian or other suitable protein expression systems, including but not limited to heteromeric molecules which employ receptors, ligands, and catalytic components such as enzymes or catalytic antibodies.

#### MATERIALS AND METHODS

gp130 in the absence of the appropriate  $R\alpha$ ).

Genetic engineering of heteromeric immunoglobulin heavy/light chain soluble receptor-based ligand traps for IL-6.

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The IL-6 traps described here were engineered using human gp130, human IL-6  $\alpha$ -receptor (IL-6R $\alpha$ ), the constant region of the heavy chains (C $\gamma$ ) of human IgG1 (Cγ1) (Lewis et al., Journal of Immunology 151:2829-2838 (1993) or IgG4 (Cy4) with or without a join-region (J), and the constant 5 regions of kappa ( $\kappa$ ) and lambda ( $\lambda$ ) (Cheung, et al., Journal of Virology 66:6714-6720 (1992) light chains of human immunoglobulin (Ig), also with or without a different j-peptide (j). This design takes advantage of the natural ability of the C $\gamma$  domain to heterodimerize with  $\kappa$  or  $\lambda$  light chains. The heterodimerization of Cy with the light chain occurs between the CH1 10 domain of Cy and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. We reasoned that, like the Fc domain of human IgG1, the combination of Cy with CL could be used to produce disulfide linked heteromeric proteins comprised of the extracellular domain of gp130 on 15 one chain and the extracellular domain of IL-6Ra on the other chain. Like their Fc-based counterparts, such proteins were postulated to be high affinity ligand traps for IL-6 and as a result to inhibit the interaction of IL-6 with the native receptor on IL-6-responsive cells, thus functioning as IL-6 antagonists. Furthermore, constructs employing the full length Cy region 20 would, much like antibodies, form homodimers of the Cy chain, giving rise to antibody-like molecules comprising of two "light chains" and two "heavy chains" (Figure 8). The potential advantage of this design is that it may more closely mimic the IL-6•IL-6Rα•gp130 complex and may display a higher affinity for the ligand than comparable single heterodimers. An 25 additional design is incorporated by using truncated versions of Cy, comprised only of the CH1 domain. These will form heterodimeric molecules with receptor-k fusion proteins, and will thus resemble the Fab fragment of antibodies.

All the soluble receptor-Ig chimeric genes may be engineered in plasmid vectors including, but not limited to, vectors suitable for mammalian expression (COS monkey kidney cells, Chinese Hamster Ovary cells [CHO], and ras-transformed fibroblasts [MG-ras]) and include a Kozak sequence 5 (CGC CGC CAC CAT GGT G) at the beginning of each chimeric gene for efficient translation. Engineering was performed using standard genetic engineering methodology. Each construct was verified by DNA sequencing, mammalian expression followed by western blotting with suitable antibodies, biophysical assays that determine ligand binding and 10 dissociation, and by growth inhibition assays (XG-1, as described later). Since the domains utilized to engineer these chimeric proteins are flanked by appropriate restriction sites, it is possible to use these domains to engineer other chimeric proteins, including chimeras employing the extracellular domains of the receptors for factors such as IL-1, IL-2, IL-3, IL-15 4, IL-5, GM-CSF, LIF, IL-11, IL-15, IFNγ, TGFβ, and others. The amino acid coordinates for each component utilized in making the IL-6 traps are listed below (Note: numbering starts with the initiating methionine as #1; long sequences are listed using the single letter code for the twenty amino acids):

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#### (a) Constructs employing human gp130:

- (i) **gp130-Cγ1** was engineered by fusing in frame the extracellular domain of gp130 (amino acids 1 to 619) to a Ser-Gly bridge, followed by the 330 amino acids which comprise Cγ1 and a termination codon (Figure 9).
- 25 (ii) **gp130-J-C**γ**1** was engineered in the same manner as gp130-Cγ1 except that a J-peptide (amino acid sequence: GQGTLVTVSS) was inserted between the Ser-Gly bridge and the sequence of Cγ1 (see Figure 9).
  - (iii)  $gp130\Delta3fibro-C\gamma1$  was engineered by fusing in frame the extracellular domain of gp130 without its three fibronectin-like domains (Figure 10).
- 30 The remaining part of this chimeric protein is identical to gp130-Cγ1.

(iv) gp130-J-CH1 was engineered in a manner identical for that described for gp130-Cγ1, except that in place of the Cγ1 region only the CH1 part of Cγ1 has been used (Figure 11). The C-terminal domain of this construct includes the part of the hinge that contains the cysteine residue
5 responsible for heterodimerization of the heavy chain of IgG with a light chain. The part of the hinge that contains the two cysteines involved in Cγ1 homodimerization has been deleted along with the CH2 and CH3 domains.

- (v) gp130-Cγ4 was engineered in a manner identical to that described for gp130-Cγ1, except that Cγ4 was used in place of Cγ1 (Figure 12). In addition, an RsrII DNA restriction site was engineered at the hinge region of the Cγ4 domain by introducing two silent base mutations. The RsrsII site allows for other desired genetic engineering manipulations, such as the construction of the CH1 equivalent of gp130-Cγ4.
- 15 (vi) **gp130-**κ was engineered in a manner identical to that described for gp130-Cγ1, except that the constant region of the κ light chain of human Ig was used in place of Cγ1 (Figure 13).
  - (vi) gp130-J- $\kappa$  was engineered in a manner identical to that described for gp130-J- $\kappa$ , except that a j-peptide (amino acid sequence: TFGQGTKVEIK) was inserted between the Ser-Gly bridge and the  $\kappa$ -region.
  - (viii)  $gp130-\lambda$  was engineered in a manner identical to that described for  $gp130-C\gamma1$ , except that the constant region of the  $\lambda$  light chain (Cheung, et al., Journal of Virology 66:6714-6720 (1992) of human Ig was used in place of  $C\gamma1$  (Figure 14).

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- (b) Constructs employing human IL-6Ra:
- (i) IL6R $\alpha$ -C $\gamma$ 1 was engineered by fusing in frame amino acids 1 to 358 of IL-6R $\alpha$  (Yamasaki et al., Science 241:825-828 (1988), which comprise the

extracellular domain of IL-6R $\alpha$  (Figure 15), to an Ala-Gly bridge, followed by the 330 amino acids which comprise C $\gamma$ 1 and a termination codon.

- (ii) **IL6R**α-κ was engineered as described for IL6Rα-Cγ1, except that the κ-domain (Figure 13) utilized for gp130-κ was used in place of Cγ1.
- 5 (iii) **IL6R** $\alpha$ -j- $\kappa$  was engineered as described for IL6R $\alpha$ - $\kappa$  except that the j-peptide described for gp130-j- $\kappa$  was placed between the Ala-Gly bridge and the  $\kappa$ -domain.
  - (iv) Three additional constructs, IL6Rα313-Cγ1, IL6Rα313-κ, and IL6Rα313-j-κ, were engineered as using a truncated form of IL-6Rα comprised of amino acids 1 to 313 (Figure 16). Each of these constructs were made by fusing in frame IL6Rα313 with a Thr-Gly bridge followed by the Cγ1, κ-, and j-κ-domains described above. These constructs were engineered in order to complement the gp130Δ3fibro-derived constructs.

# 15 Expression and purification of ligand traps

To produce covalently linked heterodimers of soluble gp130 and soluble IL-6Rα, gp130-Ig chimeric proteins were co-expressed with appropriate IL-6Rα-Ig chimeric proteins in complementing pairs. Co-expression was achieved by co-transfecting the corresponding expression vectors into suitable mammalian cell lines, either stably or transiently. The resulting disulfide-linked heterodimers were purified from conditioned media by several different methods, including but not limited to affinity chromatography on immobilized Protein A or Protein G, ligand-based affinity chromatography, ion exchange, and gel filtration.

An example of the type of methods used for purification of a heavy/light receptor fusion protein is as follows: gp130-Cγ1•IL-6Rα-κ was expressed in COS cells by co-transfecting two different vectors, encoding gp130-Cγ1 and

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IL-6R $\alpha$ - $\kappa$  respectively. Serum-free conditioned media (400 ml) were collected two days post-transfection and C $\gamma$ 1-bearing proteins were purified by affinity chromatography over a 1ml Protein A Sepharose (Pharmacia). The material generated in this step was further purified by a second affinity chromatography step over a 1 ml NHS-activated Sepharose (Pharmacia) which was derivatized with recombinant human IL-6, in order to remove gp130-C $\gamma$ 1 dimer from gp130-C $\gamma$ 1 •IL-6R $\alpha$ - $\kappa$  complexes (the gp130-C $\gamma$ 1 dimer does not bind IL-6). Proteins generated by this method were more than 90% pure, as evidenced by SDS-PAGE followed by silverstaining (Figure 17). Similar protocols have been employed successfully towards the purification of other heavy/light receptor heterodimers.

#### RESULTS

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15 <u>Biological activity of immunoglobulin heavy/light chain receptor fusion antagonists</u>

The purified ligand traps were tested for their ability to bind IL-6 in a

variety of different assays. For example, the dissociation rate of IL-6 bound to the ligand trap was measured in parallel with the dissociation rate of IL-20 6 from the anti-IL-6 monoclonal neutralizing antibody B-E8 [Brochier, et al., Int. J. Immunopharmacology 17:41-48 (1995), and references within]. An example of this type of experiment is shown in Figure 18. In this experiment 20 pM ¹²⁵I-IL-6 (1000 µCi/mmol; Amersham) was preincubated with 500 pM of either gp130-Cy1  $\bullet$  IL-6Ra- $\kappa$  or mAb B-E8 for 20 25 hours. At this point a 1000-fold excess (20 nM) of "cold" IL-6 was added. Periodically, aliquots of the reaction were removed, the ligand trap or B-E8 were precipitated with Protein G-Sepharose, and the number of cpm of 125I-IL-6 that remained bound was determined. Clearly, the dissociation rate of human  $^{125}\text{I-IL}6$  from the ligand trap was very slow - after three 30 days, approximately 75% of the initial counts were still bound to the ligand

trap. In contrast, less than 5% of the counts remained associated with the antibody after three days. This result demonstrates that the dissociation rate of the ligand from these ligand traps is very slow.

5 In a different set of experiments the ability of the ligand traps to multimerize in the presence of ligand was tested. An example of this is shown in Figure 19. IL-6-induced association of gp130-Fc•IL-6Rα-Fc with gp130-CH1•IL-6Rα-κ was determined by testing whether gp130-CH1•IL- $6R\alpha$ - $\kappa$ , which does not by itself bind Protein A, could be precipitated by 10 Protein A-Sepharose in the presence of gp130-Fc•IL-6Rα-Fc in an IL-6depended manner (Figure 9). Precipitation of gp130-CH1•IL-6Rα-κ by Protein A-Sepharose was determined by western blotting with an antikappa specific HRP conjugate, which does not detect gp130-Fc•IL-6Rα-Fc. gp130-CH1•IL-6Rα-κ could be precipitated by Protein A-Sepharose only 15 when both gp130-Fc•IL-6Rα-Fc and IL-6 were present. This result conclusively indicates that IL-6 can induce ligand trap multimerization, and further indicate that the ligand trap can mimic the hexameric cytokine•Rα•signal transducer complex (Figure 1). Ligand-induced multimerization may play a significant role in the clearance of cytokine•ligand trap complexes in vivo. 20

The biological activity of the different ligand traps may be further tested in assays which measure ligand-depended cell proliferation. Several cell proliferation assays exist for IL-6 and they employ cell lines such as B9, CESS, or XG-1. An example of this type of assay using the XG-1 cell line is presented below: XG-1 is a cell line derived from a human multiple myeloma (Zhang, et al., Blood 83:3654-3663 (1994). XG-1 depends on exogenously supplied human IL-6 for survival and proliferation. The EC50 of IL-6 for the XG-1 line is approximately 50 pmoles/ml. The ability of several different IL-6 traps to block IL-6-depended proliferation of XG-1

cells was tested by incubating increasing amounts of purified ligand traps with 50 pg/ml IL-6 in XG-1 cultures. The ligand traps which were tested had been expressed and purified by methods similar to those described above. All of the ligand traps tested were found to inhibit IL-6-dependent proliferation of XG-1 in a dose dependent manner (Figure 20). Of the five different traps tested gp130-Cγ1•IL-6Rα-κ was the most active and essentially display the same neutralizing activity towards IL-6 as the antibody B-E8. As little as a 10-fold molar excess of either gp130-Cγ1•IL- $6R\alpha$ -κ or B-E8 completely blocked the activity of IL- 6 (a reading of A570-650 = 0.3 AU corresponds to no proliferation of the XG-1 cells). At a 100fold molar excess all of the ligand traps tested completely blocked the activity of IL-6. This observed inhibition is highly selective as neither a gp130-Fc•CNTFRα-Fc ligand trap which blocks CNTF activity, nor gp130-Fc homodimer exhibit any blocking activity towards IL-6 even when used at a 1000-fold molar excess over IL-6 (data not shown). This data demonstrates that the heteromeric immunoglobulin heavy/light chain receptor-based ligand traps function as selective high affinity antagonists of their cognate ligand.

# 20 EXAMPLE 5 - CLONING OF FUSION POLYPEPTIDE COMPONENTS

The extracellular domains of the human cytokine receptors were obtained by standard PCR techniques using tissue cDNAs (CLONTECH), cloned into the expression vector, pMT21 (Genetics Institute, Inc.), and the sequences were sequenced by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). For the IL-4Rα, nucleotides 241 through 868 (corresponding to the amino acids 24-231) from the Genbank sequence, X52425, were cloned. For the IL-2Rγ, nucleotides 15 through 776 (corresponding to amino acids 1-233) from the Genbank sequence, D11086, were cloned. For the IL-6Rα, nucleotides 52 through 1044 (corresponding

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to the amino acids 1-331) from the Genbank sequence, X52425, were cloned. For gp130, nucleotides 322 through 2112 (corresponding to the amino acids 30-619) from the Genbank sequence, M57230, were cloned. For the IL-1RAcP, nucleotides 1 through 1074 (corresponding to the amino acids 1-358) from the Genbank sequence, AB006357, were cloned. For the IL-1RI, nucleotides 55 through 999 (corresponding to the amino acids 19-333) from the Genbank sequence, X16896, were cloned.

# EXAMPLE 6 - PRODUCTION OF FUSION POLYPEPTIDES (CYTOKINE TRAPS)

The nucleotide sequences encoding the cytokine traps were constructed from the individual cloned DNAs (described *supra*) by standard cloning and PCR techniques. In each case, the sequences were constructed in frame such that the sequence encoding the first fusion polypeptide component was fused to the sequence encoding the second fusion polypeptide component followed by an Fc domain (hinge, CH2 and CH3 region of human IgG1) as the multimerizing component. In some cases extra nucleotides were inserted in frame between sequences encoding the first and second fusion polypeptide components to add a linker region between the two components (See Figure 21A - Figure 21D - trap 424; Figure 24A - Figure 24F - trap 412; and Figure 26A - Figure 26E - trap 569).

For the IL-4 traps, 424 (Figure 21A - Figure 21D), 603 (Figure 22A - Figure 22D) and 622 (Figure 23A - Figure 23D), the IL-2Rγ component is 5′, followed by the IL4Rα component and then the Fc component. For the IL-6 traps, 412 (Figure 24A - Figure 24F) and 616 (Figure 25A - Figure 25F), the IL-6Rα component is 5′ followed by the gp130 component and then the Fc domain. For the IL-1 trap 569 (Figure 26A - Figure 26E), the IL-1RAcP component is 5′ followed by the IL-1RI component and then the Fc domain. The final constructs were cloned into the mammalian expression vector pCDNA3.1 (STRATAGENE).

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In the 569 sequence (Figure 26A - Figure 26E), nucleotides 1-1074 encode the IL1RAcP component, nucleotides 1075 -1098 encode a linker region, nucleotides 1099-2043 encode the IL1RI component and nucleotides 2044-2730 encode the Fc domain.

In the 412 sequence (Figure 24A - Figure 24F), nucleotides 1-993 encode the IL6Rα component, nucleotides 994-1023 encode a linker region, nucleotides 1024-2814 encode the gp130 component and nucleotides 2815-3504 encode the Fc domain.

In the 616 sequence (Figure 25A - Figure 25F), nucleotides 1-993 encode the IL6Rα component, nucleotides 994-2784 encode the gp130 component and nucleotides 2785-3474 encode the Fc domain.

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In the 424 (Figure 21A - Figure 21D) and 622 (Figure 23A - Figure 23D) sequences, nucleotides 1-762 encode the IL2R $\gamma$  component, nucleotides 763-771 encode a linker region, nucleotides 772-1395 encode the IL4R $\alpha$  component and nucleotides 1396-2082 encode the Fc domain.

- Finally, in the 603 sequence (Figure 22A Figure 22D), nucleotides 1-762 encode the IL2R $\gamma$  component, nucleotides 763-1386 encode the IL4R $\alpha$  component and nucleotides 1387-2073 encode the Fc domain.
- DNA constructs were either transiently transfected into COS cells or stably transfected into CHO cells by standard techniques well known to one of skill in the art. Supernatants were collected and purified by Protein A affinity chromatography and size exclusion chromatography by standard techniques. (See for example Harlow and Lane, Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, 1988).

### EXAMPLE 7: IL-4 BIOASSAY PROTOCOL USING TF-1 (ATCC) CELLS.

# Reagents and Equipment Needed

## 5 MTT Dve Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128) Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca+2, Mg+2.

10 Sterile filter and store aliquoted at -20°C

#### Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml d $H_2$ 0, 50 ml Dimethyl Formamide, and 850  $\mu$ l concentrated HCl.

Filter sterilize with a 0.45µm filter unit.

Store at room temperature

#### TF-1 cell Growth Medium:

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RPMI 1640, 10% FBS, Pen/Strep, 2mM L-glutamine

#### Other:

25 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon #3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100µl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

#### Assay Protocol

# A. Preparation of Assay plates

Prepare sterile 96 well tissue culture plates to contain 50μl of growth medium per well with various concentrations of IL-4 and 10nM IL-4 antagonist. This can be done by preparing a working dilution of IL-4 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-4. Add 25μl of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25μl of growth medium without IL-4 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25μl to a triplicate set of IL-4 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H.

2. As a positive control, leave one set with no antagonist. These wells will contain IL-4 and media only.

3. Incubate the plate for 1-2 hours at 37°C in a humidified 5% CO₂
 20 incubator before preparing cells to be used for assay.

# B. Preparation of Cells

- 4. Wash cells twice by centrifugation in assay medium free of growth factor.
  - 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of  $8 \times 10^5$ /ml in assay medium.
- 6. Dispense 50μl of the cell suspension (40,000 cells) into all wells of the plates. Total volume should now be 100μl/well.

7. Incubate the plate at  $37^{\circ}$ C for 68 hours in a humidified 5% CO₂ incubator.

## C. Color Development

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- 8. After incubating for 68 hours, add 15µl of the MTT dye solution to each well.
- 9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

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- 10. After 4 hours, add 100µl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.
- 15 11. Record the absorbance at 570/650nm.

#### **RESULTS**

Figure 27 shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2Rγ-scb-IL4Rα-FcΔC1, is several orders of magnitude better as an IL-4 antagonist than IL4RαFcΔC1 alone in the TF1 cell bioassay.

Figure 28 shows that the IL-4 trap designated 4SC375 shows antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 which is a fusion polypeptide of IL-2R $\gamma$ -IL4R $\alpha$ -Fc $\Delta$ C1 having the IL-2R $\gamma$  component flush with the IL-4R $\alpha$  component.

# **EXAMPLE 8: IL-6 BIOASSAY PROTOCOL USING XG-1 CELLS**

## 30 Reagents and Equipment Needed

#### MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca⁺², Mg⁺².

Sterile filter and store aliquoted at -20°C

#### Solubilization Solution:

10 For 1000 ml, combine 100 g SDS, 950 ml d $H_2$ 0, 50 ml Dimethyl Formamide, and 850  $\mu$ l concentrated HCl.

Filter sterilize with at 0.45µm filter unit.

Store at room temperature

#### 15 Assay Medium:

RPMI 1640, 10%FBS, Pen/Strep, 2mM L-glutamine, 50μM mercaptoethanol.

#### 20 Other:

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0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100µl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

#### Assay Protocol

#### A. Preparation of Assay plates

1. Prepare sterile 96 well tissue culture plates to contain 50µl of growth medium per well with various concentrations of IL-6 and 10nM IL-6 antagonist. This can be done by preparing a working dilution of IL-6 that is

4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-6. Add 25µl of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25µl of growth medium without IL-6 to row H.

- Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25µl to a triplicate set of IL-6 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H. A typical IL-6 titration starts at 200ng/ml down to 3.1ng/ml.
- 10 2. As a positive control, leave one set with no antagonist. These wells contain IL-6 and media in place of antagonist.
  - 3. Incubate the plate 1-2 hours at 37oC in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

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## B. Preparation of Cells

4. Wash cells twice by centrifugation (5 min at 1000RPM) in assay medium free of growth factor.

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- 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of  $8 \times 10^5$ /ml in assay medium.
- 6. Dispense 50µl of the cell suspension (40000 cells) into all wells of the plates. Total volume should now be 100µl/well.
  - 7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

# 30 <u>C. Color Development</u>

8. At 68 hours add  $15\mu l$  of the dye solution to each well.

9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

10. After 4 hours, add 100µl of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

11. Record the absorbance at 570/650nm.

#### **RESULTS**

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Figure 29 shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc $\Delta$ C1) described in Figure 24A - Figure 24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

#### 15 EXAMPLE 9: MRC5 BIOASSAY FOR IL1 TRAPS

MRC5 human lung fibroblast cells respond to IL-1 by secreting IL-6 and thus were utilized to assay the ability of IL-1 traps to block the IL-1-dependent production of IL-6. IL1 Trap 1SC569 (Figure 26A - Figure 26E) was tested against IL-1-RI.Fc which is the extracellular domain of the IL-1 Type I receptor fused to an Fc domain.

MRC5 cells are suspended at  $1 \times 10^5$  cells per ml in medium and 0.1 ml of cells are plated (10,000 cells per well) into the wells of a 96 well tissue culture plate. Plates are incubated for 24 hours at 37°C in a humidified 5% CO₂ incubator.

IL-1 trap and recombinant human IL-1 at varying doses are pre-incubated in a 96 well tissue culture dish and incubated for 2 hours at 37°C. 0.1 ml of this mixture is then added to the 96 well plate containing the MRC5 cells such that the final concentration of IL-1 Trap is 10nM and the final

concentrations of the IL-1 ranges from 2.4 pM to 5nM. Control wells contain trap alone or nothing.

Plates are then incubated at 37°C for 24 hours in a humidified 5% CO₂ incubator. Supernatant is collected and assayed for levels of IL-6 using R&D Systems Quantikine Immunoassay Kit according to the manufacturer's instructions.

#### **RESULTS**

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Figure 30 shows that the trap 569 (Figure 26A - Figure 26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1. At a concentration of 10nM, the trap 569 is able to block the production of IL-6 up to an IL-1 concentration of 3nM. In contrast, the IL-1RI.Fc is a much poorer antagonist of IL-1. It is only able to block the effects of IL-1 up to about 10-20 pM. Thus, the trap 569 is approximately 100x better at blocking IL-1 than IL1RI.Fc.

#### EXAMPLE 10 - CONSTRUCTION OF IL-13/IL-4 SINGLE CHAIN TRAPS

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1. To create the IL-13/IL-4 dual trap designated IL-4R $\alpha$ .IL-13R $\alpha$ 1.Fc, the human IL-4R $\alpha$  extracellular domain (corresponding to nucleotides #1-693 of Figure 31A - Figure 31G) and the human IL-13R $\alpha$ 1 extracellular domain (corresponding to nucleotides #700-1665 of Figure 31A - Figure 31G) were amplified by standard PCR techniques and ligated into an expression vector pMT21 which contained the human Fc sequence (corresponding to nucleotides #1671-2355 of Figure 31A - Figure 31G), thus creating a fusion protein consisting of the IL-4R $\alpha$ , IL-13R $\alpha$ 1, and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a two amino acid linker (corresponding to nucleotides #694-699 of Figure 31A - Figure 31G) with the amino acid sequence SerGly was constructed in frame

between the IL-4Rα and the IL-13Rα1 and a two amino acid linker (corresponding to nucleotides #1666-1671 of Figure 31A - Figure 31G) with the amino acid sequence ThrGly was constructed in frame between the IL-13Rα1 and the Fc portion. All sequences were sequence-verified by standard techniques. The IL-4Rα.IL-13Rα1.Fc coding sequence was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

2. To create the IL-13/IL-4 dual trap designated IL-13Rα1.IL-4Rα.Fc, the IL-10 13Rα1 extracellular domain (corresponding to nucleotides #1-1029 of Figure 32A - Figure 32G) and the human IL-4Rα (corresponding to nucleotides # 1060-1692 of Figure 32A - Figure 32G) were amplified by standard PCR techniques and ligated into the expression vector pJFE14, which contains the human Fc sequence (corresponding to nucleotides #1699-2382 of Figure 32A - Figure 32G) to create a fusion protein consisting 15 of the IL-13Rα1, IL-4Rα, and the hinge, CH2 and CH3 region of human IgG1 from the N to C terminus. In addition, a ten amino acid linker with the amino acid sequence GlyAlaProSerGlyGlyGlyGlyArgPro (corresponding to nucleotide #1030-1059 of Figure 32A - Figure 32G) was 20 constructed in frame between the IL-13R $\alpha$ 1 and the IL-4R $\alpha$  and a two amino acid linker (corresponding to nucleotides #1693-1698 of Figure 32A -Figure 32G) with the amino acid sequence SerGly was constructed in frame between IL-4Rα and the Fc portion. All sequences were sequence-verified using standard techniques. The coding sequence of IL-13R $\alpha$ 1.IL-4R $\alpha$ .Fc 25 was then subcloned into the expression vector pCDNA3.1 (Stratagene) using standard molecular biology techniques.

EXAMPLE 11: EXPRESSION OF IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc

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Large scale (1L) cultures of the pCAE801 (the DNA vector construct encoding IL-4Rα.IL-13Rα1.Fc) and pCAE802 (the DNA plasmid construct encoding IL-13Rα1.IL-4Rα.Fc) in DH10B cells were grown overnight in LB + ampicillin and the plasmid DNA was extracted using a Qiagen Endofree Mega Kit following the manufacturer's protocol. The concentration of the purified plasmid DNA was determined in a UV spectrophotometer and fluorometer. The plasmid DNA was also verified by digestion of aliquots with BbsI, XmnI and NcoI restriction enzymes. All restriction enzyme digest fragments corresponded to the predicted sizes in a 1% agarose gel.

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Forty 15 cm petri plates were seeded with CHO-K1/E1A cells at a density of 4 x 10° cells/plate. Plating media was Gibco Ham's F-12 w/10% Hyclone Fetal Bovine Serum (FBS) + penicillin/streptomycin and supplemented with glutamine. The following day each plate was transfected with 6 µg of pCAE801, or pCAE802, using Gibco Optimem and Gibco Lipofectamine in 12 ml volume, following the manufacturer's protocol. Four hours after adding the transfection mix to the cells 12 ml/plate of Optimem w/ 10% FBS was added. Plates were incubated at 37°C in a 5% CO₂ incubator overnight. The following day the media was removed from each plate and 25 ml expression media (Gibco CHO-S-SFM II w/ glutamine + 1mM sodium butyrate) was added. The plates were incubated at 37°C for 3 days.

After 3 days of incubation the media was removed from each plate and centrifuged at 400 rpm in a swinging bucket rotor to pellet cells. The supernatant was decanted into sterile 1L bottles and expressed protein was purified as described *infra*.

# EXAMPLE 12: PURIFICATION OF IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc PROTEIN FROM CULTURE MEDIA

#### 1. Purification of IL-4Rα.IL-13Rα1.Fc.

Human IL-4Rα.IL-13Rα1.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described supra. 5 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (y chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield ranged from 5.8 to 9.2 mg (average of 7.5 mg) per liter of conditioned media. Complete $^{\text{TM}}$  protease inhibitor tablets (Roche 10 Diagnostics Corp.) were dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 µm pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap® Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The 15 column was extensively washed with PBS buffer to remove nonspecifically bound proteins from the column. IL-4Rα.IL-13Rα1.Fc_was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, 20 pH 7.4 at 4°C. The recovery from Protein A purification was 6.8 mg (73%). IL-4Rα.IL-13Rα1.Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were 25 assessed from a Coomassie stained non-reduced and reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were conservatively pooled to reduce the amount of aggregated protein. The overall yield was 51% (4.4 mg) with a purity of 97% as judged by SDS-PAGE. Purified IL-4Rα.IL-13Rα1.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-30 12% Bis-Tris), analytical size exclusion chromatography (Tosohaas

TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4R (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

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#### 2. Purification of IL-13Rα1.IL-4Rα.Fc

Human IL-13Rα1.IL-4Rα.Fc was transiently expressed in CHO cells and supernatants were harvested from plate transfections as described supra. 10 Expression of the secreted protein was determined by a sandwich ELISA using goat anti-hIgG (y chain specific; Sigma 1-3382) and goat anti-hIgG (Fc specific)-FITC conjugate (Sigma F9512) capture and report antibodies, respectively. The yield was 8.8 mg per liter of conditioned media. CompleteTM protease inhibitor tablets (Roche Diagnostics Corp.) were 15 dissolved into the media (1 tablet/L). The conditioned media was sterile filtered (0.22 µm pore size) prior to loading onto a pre-equilibrated, 5 mL HiTrap® Protein A affinity column (Amersham Pharmacia Biotech) in Dulbecco's PBS buffer (Life Technologies), pH 7.4 at 4°C. The flow rate was ~1-2 mL/min. The column was extensively washed with PBS buffer to 20 remove nonspecifically bound proteins from the column. IL-13Rα1.IL-4Rα.Fc was eluted using 20 mM sodium citrate, 150 mM NaCl, pH 3.5. The eluate was immediately neutralized by titrating with 1 M Tris-OH. The fractions containing protein were pooled and immediately dialyzed in PBS buffer, pH 7.4 at 4 °C. The recovery from Protein A purification was 3.8 mg 25 (43%). IL-13Rα1.IL-4Rα.Fc was further purified by size exclusion chromatography using a superose 6 column (25 mL bed volume; Amersham Pharmacia Biotech) pre-equilibrated in PBS, 5% v/v glycerol, pH 7.4 at ambient temperature. The flow rate was 0.5 mL/min. Protein fractions were assessed from a Coomassie stained non-reduced and 30 reduced SDS-PAGE (Novex NuPAGE 4-12% Bis-Tris gels). Fractions were

conservatively pooled to reduce the amount of aggregated protein. The overall yield was 17% (1.5 mg) with a purity of 95% as judged by SDS-PAGE. Purified IL-13Rα1.IL-4Rα.Fc was analyzed by non-reduced and reduced SDS-PAGE (4-12% Bis-Tris), analytical size exclusion chromatography (Tosohaas TSKG4000SWXL), N-terminal sequencing, and immunoblotting with goat anti-hIgG-HRP conjugate (Promega W403B), and also mouse monoclonal anti-hIL-4Rα (R&D MAB230) followed by anti-mIgG-HRP conjugate (Promega W402B) as the secondary antibody.

# 10 EXAMPLE 13: BLOCKING OF IL-4 AND IL-13 BY IL-4Rα.IL-13Rα1.Fc AND IL-13Rα1.IL-4Rα.Fc

#### Materials and Methods

TF1 Bioassay. TF1 cells were maintained in growth media (10ng/ml GM-CSF, RPMI 1640, 10% FBS, L-glutamine, Penicillin, Streptomycin). For the bioassay, cells were washed 2 times in assay media (as above but without GM-CSF) and then plated at 2 x 10⁵ cells in 50μl of assay media. The purified IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc proteins were diluted into assay media at a concentration of 40nM. 25ul of each of the traps was added to the cells. Either IL-13 or IL-4 were diluted to 40nM in assay media and then 2-fold dilution series in assay media were made. 25μl of either IL-13 or IL-4 was then added to the wells containing the cells and the traps. Cells were then incubated at 37°C, 5% CO₂ for ~70 hrs. The extent of TF1 cell proliferation was measured by the MTS assay according to the manufacturer's protocol (Promega, Inc.).

#### **RESULTS**

The ability of the IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc traps to block both human IL-13 and human IL-4 activity was measured in the TF1

bioassay described *supra*. IL-13 stimulates proliferation of TF1 cells, with half-maximal growth at a concentration of 0.2nM. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc trap at a concentration of 10nM blocks IL-13-induced growth up to ~2nM (Figure 33). At an IL-13 concentration of ~4-5 nM the growth of TF1 cells is inhibited by 50%. TF1 cells are more sensitive to IL-4, which stimulates their proliferation with half-maximal growth at ~0.02nM. Addition of either IL-4Rα.IL-13Rα1.Fc or IL-13Rα1.IL-4Rα.Fc at a concentration of 10nM blocks IL-4-induced growth up to ~1nM (Figure 34). At an IL-4 concentration of ~3-4 nM the growth of TF1 cells is inhibited by 50%. These results show that both IL-4Rα.IL-13Rα1.Fc and IL-13Rα1.IL-4Rα.Fc can block the ability of both IL-13 and IL-4 to stimulate cellular responses.

#### EXAMPLE 14: BLOCKING OF INJECTED IL-1 BY IL-1 TRAP IN VIVO

IL-1 is a pro-inflammatory cytokine. Systemic administration of IL-1 has been shown to elicit acute responses in animals, including transient hyperglycemia, hypoinsulinemia, fever, anorexia, and increased serum levels of interleukin-6 (IL-6) (Reimers, 1998). Since mice are responsive to both murine and human IL-1, human IL-1 can be used and *in vivo* binding effects of human specific IL-1 antagonists can be evaluated. This acute mouse model was used to determine the ability of a human IL-1 trap to antagonize the *in vivo* effects of exogenously administered human IL-1. This provides a rapid indication of *in vivo* efficacy of the human IL-1 trap and can be used as an assay to help molecule selection.

#### **Experimental Design:**

Mice were given subcutaneous injections of human IL-1 (0.3 μg/kg).

Twenty-four hours prior to human IL-1 injection, the animals were pretreated with either vehicle or 150-fold molar excess of human IL-1 trap (0.54 mg/kg). Two hours prior to sacrifice (26 hrs), the mice were given a

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second injection of human IL-1 (0.3  $\mu$ g/kg). Blood samples were collected at various time points and sera were assayed for IL-6 levels.

#### **RESULTS**

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Exogenous administration of human IL-1 resulted a dramatic induction of serum IL-6 levels. At 150-fold molar excess, the human IL-1 trap completely blocked the IL-6 increase (Figure 35). Furthermore, the effects of the human IL-1 trap persisted for at least another 24 hours, preventing an IL-6 increase even when IL-1 was re-administered (Figure 35). Such long-lasting efficacy suggests that daily injection of an IL-1 trap may not be necessary for chronic applications.

EXAMPLE 15: EVALUATING THE ABILITY OF AN IL-4 TRAP TO

BLOCK THE PHYSIOLOGICAL RESPONSES TO HUMAN IL-4 IN

CYNOMOLOGUS MONKEYS.

Systemic administration of human IL-4 elicits systemic responses in Cynomologus monkeys (Gundel et al., 1996). Thus, the effectiveness of the IL-4 trap in blocking human IL-4 can be demonstrated by measuring these responses.

#### **Experimental Design:**

The experiment consisted of 3 parts: human IL-4 + vehicle (part 1), human IL-4 + IL-4 Trap (part 2), and human IL-4 + vehicle (part 3).

Human IL-4 (25 μg/kg) was injected subcutaneously twice daily for 4 days and IL-4 Trap (8 mg/kg) and vehicle were given intravenously daily for 5 days, beginning 1 day prior to human IL-4 administration. Whole blood was collected daily for flow cytometry analysis for CD16 and plasma was obtained to assay for the cytokine monocyte chemotactic protein 1 (MCP-1).

CD16 and MCP-1 are markers of IL-4-mediated inflammation in both humans and monkeys.

#### **RESULTS**

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In the presence of human IL-4, MCP-1 increased 2.5-fold and was significantly blocked by the IL-4 Trap (Figure 36A). Similarly, the decrease in the percent of CD16 positive lymphocytes in peripheral blood was attenuated by the IL-4 trap (Figure 36B). After a rest period, the monkeys were re-injected with human IL-4 and the responsiveness of the animals to human IL-4 was re-confirmed (Figures 36A and 36B), suggesting that inhibition of the MCP-1 and CD 16 responses is specifically mediated by the IL-4 trap.

## 15 EXAMPLE 16: THE EFFECTS OF IL-4 TRAP ON 1L-4-INDUCED IgE SECRETION.

It has been shown that injection of anti-mouse IgD antibody stimulates an IL-4-mediated IgE increase in normal mice. This model has been widely used to evaluate IL-4 antagonists, such as soluble IL-4 receptor and anti-IL-4 monoclonal antibodies (Sato et al., 1993). We decided to use this model to evaluate the ability if the IL-4 trap to block IL-4-mediated increases of IgE.

### 25 Experimental design:

BALB/C mice injected with anti-mouse IgD (100µl/mouse, s.c.) were randomly divided into 3 groups. Each received (on days 3-5) either vehicle, murine IL-4 trap (1 mg/kg, s.c.), or a monoclonal antibody to mouse IL-4 (1 mg/kg, s.c.). Serum was collected at various time points and assayed for IgE levels.

#### **RESULTS**

Treatment with the murine IL-4 trap or the mouse IL-4 antibody both significantly antagonized the IL-4-mediated IgE increase in this mouse model (Figure 37). This suggests that the murine IL-4 trap binds murine IL-4 and antagonizes physiological responses elicited by endogenous IL-4 *in vivo*.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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#### WE CLAIM:

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1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:

- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
- b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
- c) a nucleotide sequence encoding a third fusion polypeptide
   15 component comprising the amino acid sequence of a multimerizing component.
  - 2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component.
    - 3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component.

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4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor and cardiotrophin-1

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

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6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

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7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

- The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF-β/BMP family selected from the group consisting of TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.
- 9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18 and MIF.
- 30 10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

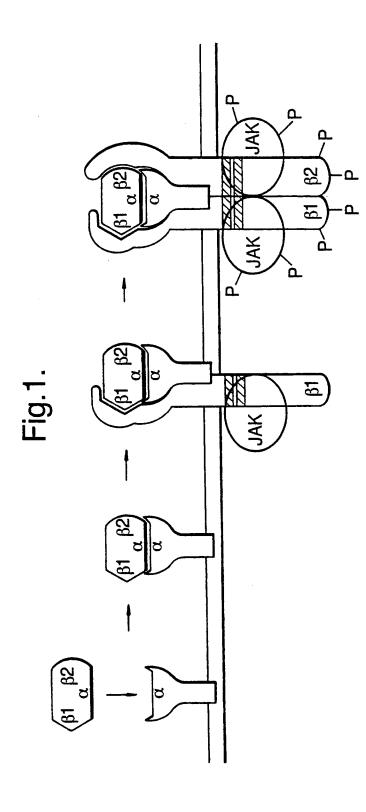
5

- 12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.
- 13. A composition capable of binding a cytokine to form a
   10 nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.
  - 14. The composition of claim 13, wherein the multimer is a dimer.
- 15. A vector which comprises the nucleic acid molecule of claim 1.
  - 16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

- 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.
- 18. The host-vector system of claim 17, wherein the suitable host cell is 25 a bacterial cell, yeast cell, insect cell, or mammalian cell.
  - 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.
- 30 20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

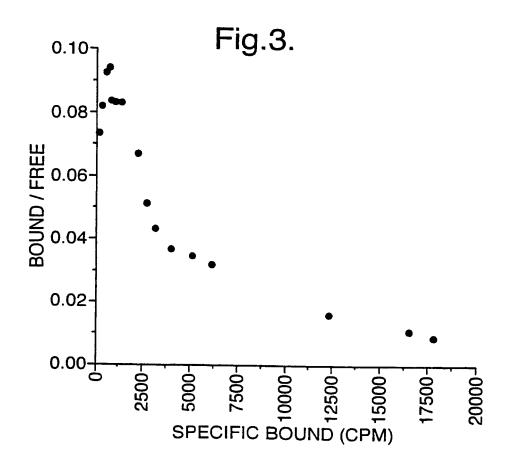
21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.

- 22. The host-vector system of claim 17, wherein the suitable host cell is a 293 cell.
  - 23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.
- 10 24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.
  - 25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.



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Fig.2. +CNTF 50 ng/ml 200 ng/ml **sCNTFR** sIL6R IL6 gp130► 1 2 3 5 6 7 9 8 10



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Fig.4.

Amino acid sequence of human gp130-Fc-His6

S	equence Ra	ange: 1 to	861			
	10	20	30	40 *	50 *	60
ħ	(VTLQTWVVQ)	Alfiflttes	TGELLDPCGYI	SPESPVVQL	HSNFTAVCVLK	EKCMDYFHV
	70 *	80	90	100	110	120
1	IANYIVWKTNI	HFTIPKEQYT	IINRTASSVTF	TDIASLNIQ	LTCNILTFGQL	EQNVYGITI
	130	140	150	160	170	180
3	SGLPPEKPKI	NLSCIVNEGK	KMRCEWDGGRE	THLETNFTL	KSEWATHKFAL	CKAKRDTPT
	190	200	210	220	230	240
5	CTVDYSTVY	FVNIEVWVEA	ENALGKVTSDH	INFDPVYKV	KPNPPHNLSVI	NSEELSSIL
	250	260	270	280	290	300
I	CLTWTNPSIK	SVIILKYNIQ	YRTKDASTWSQ	PPEDTAST	RSSFTVQDLKI	FTEYVFRIR
	310	320	330	340	350	360
(	MKEDGKGYW	SDWSEEASGI	TYEDRPSKAPS	FWYKIDPSH	TQGYRTVQLVV	vktlppfean
	370 *	380	390	400	410	420
(	SKILDYEVTL	TRWKSHLQNY	TVNATKLTVNI	TNDRYLATL	TVRNLVGKSD	AAVLTIPACD
	430 *	440	450 *	460 *	470 *	480
1	FQATHPVMDL	KAFPKDNMLW	VEWTTPRESVE	KYILEWCVL	SDKAPCITDW	QQEDGTVHRT
	490 *	500 *	510 *	520 *	530 *	540 *
•	YLRGNLAESK	CYLITVTPVY	ADGPGSPESI	KAYLKQAPPS	KGPTVRTKKV	GKNEAVLEWD
	550 *	560 *	570 *	580 *	590 *	600 *
(	QLPVDVQNGF	IRNYTIFYRT	IIGNETAVNVI	DSSHTEYTLS	SLTSDTLYMV	RMAAYTDEGG
	610 *	620 *		640 t t *	650 *	660 *
1	KDGPEFTFTT	PKFAQGEIES	GEPKSCDKTH	•	LGGPSVFLFP	PKPKDTLMIS
	670 *	680	690 *	700 *	710	
	RTPEVTCVVV	DVSHEDPEVK	FNWYVDGVEV	<u>HNAKTKPREE</u>	OYNSTYRVVS	
	730	740	750	760	770	780

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Fig.4 (Cont).

NGKEYKCKVSNKALPAPIEK TISKAKGOPREPOVYTLPPS RDELTKNOVSLTCLVKGFYP 790 800 810 820 830 840

SDIAVEWESNGOPENNYKTT PPVLDSDGSFFLYSKLTVDK SRWOOGNVFSCSVMHEALHN

850 860

HYTOKSLSLSPGK HHHHHH+

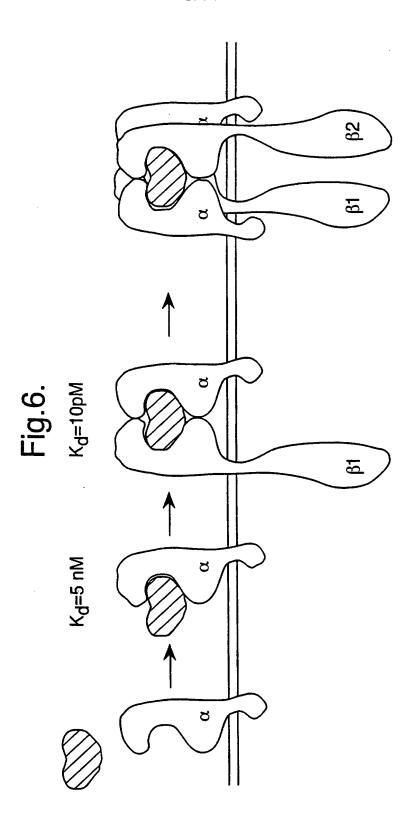
Fig.5.

The amino acid sequence of human IL-6Rα-Fc

Sequence Range: 1 to 594

10	20	30	40	50	60
MVAVGCALLA	ALLAAPGAAL	APRRCPAQEVA	ARGVLTSLPG	DSVTLTCPGVI	EPEDNATVHW
70 *	80	90	100	110	120
VLRKPAAGSHI	PSRWAGMGRR	LLLRSVQLHD	SGNYSCYRAG	RPAGTVHLLVI	OVPPEEPQLS
130	140	150	160	170	180
CFRKSPLSNVV	CEWGPRSTP	SLTTKAVLLVE	RKFQNSPAED	FQEPCQYSQES	SQKFSCQLAV
190 *	200 *	210	220	230	240
PEGDSSFYIVS	MCVASSVGS	KFSKTQTFQGC	GILQPDPPA	NITVTAVARNE	RWLSVTWQD
250 *	260 *	270	280	290	300
PHSWNSSFYRL	RFELRYRAE	RSKTFTTWMVK	CDLQHHCVIH	DAWSGLRHVVQ	* LRAQEEFGQ
310	320	330	340	350	360
GEWSEWSPEAM	GTPWTESRS	PPAENEVSTP	1QALTTNKDD	DNILFRDSAN	* \TSLPVQDAG
370 *t	380	390	400	410	420
EPKSCDKTHTC	PPCPAPELL	GGPSVFLFPPI	CPKDTLMISR	TPEVTCVVVD	SHEDPEVKF
430 *	440	450	460	470	480
NWYVDGVEVHN	IAKTKPREEO	YNSTYRVVSVI	LTVLHODWLN	GKEYKCKVSNI	KALPAPIEKT
490 *	500	510	520	530	540
ISKAKGOPREP	POVYTLPPSR	DELTKNOVSL	CLVKGFYPS	DIAVEWESNG	* OPENNYKTTP
550 *	560	570	580	590	
PVLDSDGSFFL	YSKLTVDKS	RWOOGNVFSCS	VMHEALHNH	YTOKSLSLSPO	<u> </u>





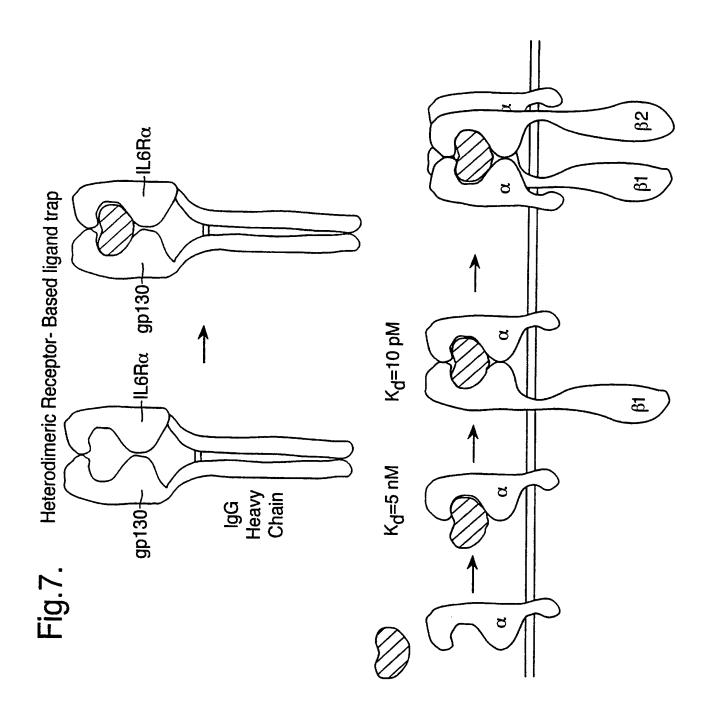
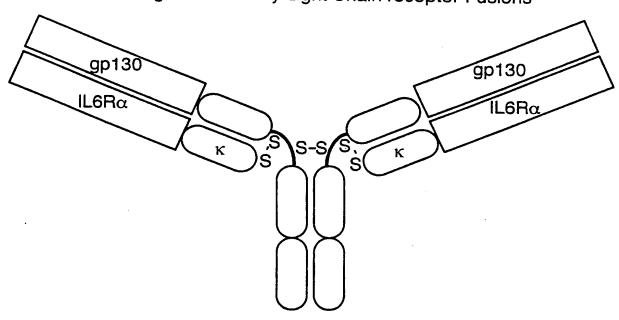


Fig.8.

Immunoglobulin Heavy/Light Chain receptor Fusions



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Fig.9.

### Amino acid sequence of gp130-Cy1

Sequence Range: 1 to	952	
10 20	30 40	50 60
MVTLQTWVVQALFIFLTTES	TGELLDPCGYISPESPVVQL	HSNFTAVCVLKEKCMDYFHV
70 80 *	90 100	110 120
NANYIVWKTNHFTIPKEQYT	IINRTASSVTFTDIASLNIQ	LTCNILTFGQLEQNVYGITI
130 140	150 160	170 180
ISGLPPEKPKNLSCIVNEGK	KMRCEWDGGRETHLETNFTL	KSEWATHKFADCKAKRDTPT
190 200	210 220	230 240
SCTVDYSTVYFVNIEVWVEA	ENALGKVTSDHINFDPVYKV	KPNPPHNLSVINSEELSSIL
250 260 * *	270 280	290 300
KLTWTNPSIKSVIILKYNIQ	YRTKDASTWSQIPPEDTAST	RSSFTVQDLKPFTEYVFRIR
310 320 *	330 340	350 360
CMKEDGKGYWSDWSEEASGI	TYEDRPSKAPSFWYKIDPSH	TQGYRTVQLVWKTLPPFEAN
370 380 * *	390 <b>4</b> 00	410 420 * *
GKILDYEVTLTRWKSHLQNY	TVNATKLTVNLTNDRYLATI	TVRNLVGKSDAAVLTIPACD
430 440 * *	450 460 * *	470 480 * *
FQATHPVMDLKAFPKDNMLW	VEWTTPRESVKKYILEWCVI	SDKAPCITDWQQEDGTVHRT
490 500 * *	510 520 * *	530 540 * *
YLRGNLAESKCYLITVTPVY	ADGPGSPESIKAYLKQAPPS	KGPTVRTKKVGKNEAVLEWD
550 560 * *	570 580 *	590 600
QLPVDVQNGFIRNYTIFYRI	IIGNETAVNVDSSHTEYTLS	S SLTSDTLYMVRMAAYTDEGG
610 620 * *	630 640	650 660
KDGPEFTFTTPKFAQGEIES	GASTKGPSVFPLAPSSKSTS	GGTAALGCLVKDYFPEPVTV
670 680 *	690 700	710 720
SWNSGALTSGVHTFPAVLOS	SGLYSLSSVVTVPSSSLGT	D TYICNVNHKPSNTKVDKKVE
730 746	750 76	770 780
PKSCDKTHTCPPCPAPELLA	G GPSVFLFPPKPKDTLMISR	T PEVTCVVVDVSHEDPEVKFN

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## Fig.9 (Cont).

790 800 810 820 830 840

WYVDGVEVHNAKTKPREEOY NSTYRVVSVLTVLHODWLNG KEYKCKVSNKALPAPIEKTI

850 860 870 880 890 900

SKAKGOPREPOVYTLPPSRD ELTKNOVSLTCLVKGFYPSD IAVEWESNGOPENNYKTTPP

910 920 930 940 950

VLDSDGSFFLYSKLTVDKSR WOOGNVFSCSVMHEALHNHY TOKSLSLSPGK*

### Fig. 10.

### Amino acid sequence of gp130\Delta3fibro

Sequence Range: 1 to 332 10 30 50 MVTLQTWVVQALFIFLTTES TGELLDPCGYISPESPVVQL HSNFTAVCVLKEKCMDYFHV 80 90 100 110 120 NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTFGQLEQNVYGITI 130 140 150 160 170 180 ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT SCTVDYSTVYFVNIEVWVEA ENALGKVTSDHINFDPVYKV KPNPPHNLSVINSEELSSIL 260 270 280 KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPFTEYVFRIR 330 CMKEDGKGYWSDWSEEASGI TYEDRPSKAPSG

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Fig. 11.

Amino acid sequence of J-CH1

Fig.12.

Sequence Range: 1 to 330

### Amino acid sequence of Cy4

10 20 60 SGASTKGPSVFPLAPCSRST SESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQ 70 80 90 100 120 SSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRV ESKYGPPCPSCPAPEFLGGP 150 160 180 SVFLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNS 220 200 TYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEM 270 280 300 TKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWO 320 330 EGNVFSCSVMHEALHNHYTQ KSLSLSLGK*

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Fig.13.

## Amino acid sequence of κ-domain

Fig.14.

### Amino acid sequence of $\lambda$ -domain:

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Fig.15.

Amino acid sequence of the soluble IL-6R $\alpha$  domain Sequence Range: 1 to 360

			500	300 2 00	<u>-</u>
60	50	40	30	20	10
*	*	*	*	*	*
PEDNATVHW	DSVTLTCPGVE	ARGVLTSLPG	APRRCPAQEVA	ALLAAPGAAL	MVAVGCALLA
120	110	100	90	80	70
*	*	*	*	*	*
VPPEEPQLS	RPAGTVHLLVD	SGNYSCYRAG	LLLRSVQLHDS	PSRWAGMGRR	VLRKPAAGSH
180	170	160	150	140	130
*	*	*	*	*	*
gkfscqlav	FQEPCQYSQES	RKFQNSPAED	SLTTKAVLLVI	VCEWGPRSTP	CFRKSPLSNV
240	230	220	210	200	190
*	*	*	*	*	*
RWLSVTWQD	NITVTAVARNP	CGILQPDPPA	KFSKTQTFQG	emcvassvgs	PEGDSSFYIV
300	290	280	270	260	250
*	*	*	*	*	*
)LRAQEEF <b>G</b> Q	DAWSGLRHVVC	KDLQHHCVIH	RSKTFTTWMVI	LRFELRYRAE	PHSWNSSFYR
360	350	340	330	320	310
*	*	*	*	*	*
ATSLPVQDAG	DNILFRDSANA	MQALTTNKDD	PPAENEVSTP	MGTPWTESRS	GEWSEWSPEA

## Fig. 16.

Amino acid sequence of the soluble IL-6ku313 domain

				_	
			315	ge: 1 to	Sequence Rai
60	50	40	30	20	10
*	*	*	*	*	*
PEDNATVHW	DSVTLTCPGVE	RGVLTSLPG	APRRCPAQEVA	LAAPGAAL	MVAVGCALLAA
120	110	100	90	80	70
*	*	*	*	*	*
VPPEEPQLS	RPAGTVHLLVD	GNYSCYRAG	LLLRSVQLHDS	RWAGMGRR	VLRKPAAGSHP
180	170	160	150	140	130
*	*	*	*	*	+
QKFSCQLAV	FQEPCQYSQES	KKFQNSPAED	SLTTKAVLLVE	EWGPRSTP	CFRKSPLSNVV
240	230	220	210	200	190
*	*	*	*	*	*
RWLSVTWQD	NITVTAVARNP	GILQPDPPA	KFSKTQTFQGC	CVASSVGS	PEGDSSFYIVS
300	290	280	270	260	250
*			*	*	*
LRAQEEFGQ	DAWSGLRHVVQ	<b>OLOHHCAIH</b>	RSKTFTTWMVK	FELRYRAE	PHSWNSSFYRL
					310

#### GEWSEWSPEAMGTTG

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Fig.17.

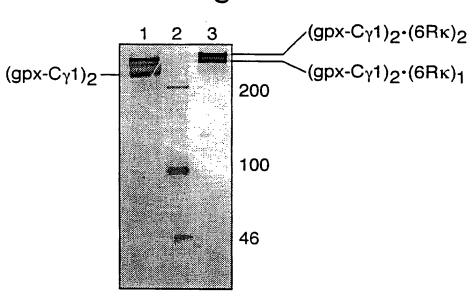
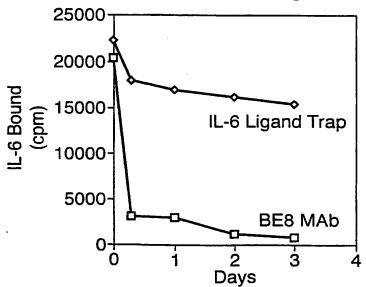
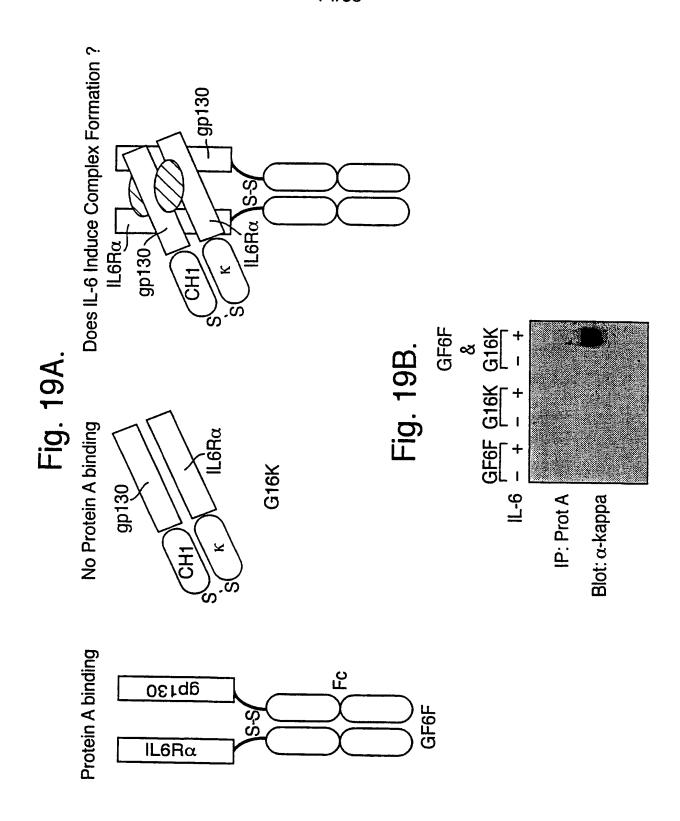


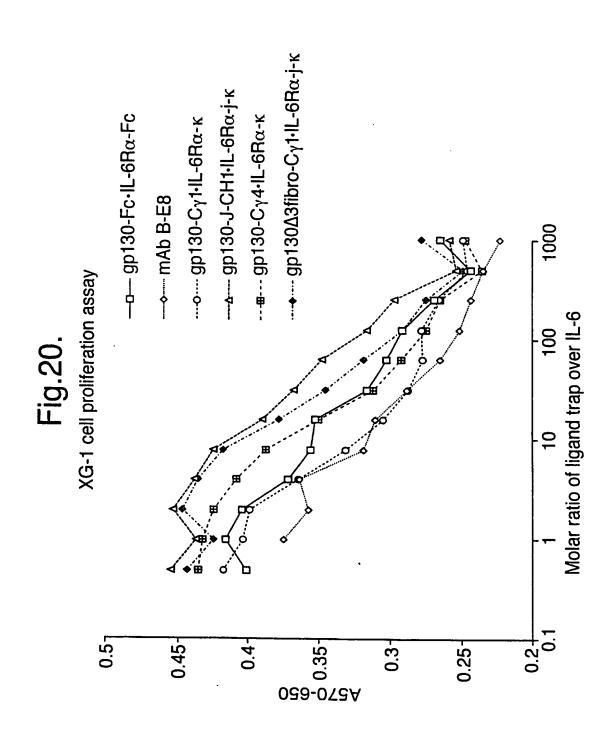
Fig. 18.

IL-6 Dissociates Slowly from the Ligand Trap









16/63 Fig.21A. 10 20 30 ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu> 50 70 80 90 * * CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly> 130 110 120 AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp> 160 170 180 TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val> 200 210 220 240 TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro> 260 270 CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn> 300 310 320 GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr> 340 350 360 370 TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe> 410 400 420 GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln> 460 470 450 480 ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu> 500 510 520 ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn> 550 560 AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC

Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

Fig.21B. 17/63

580 590 600 610 620 TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe> 640 650 660 TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg> 690 700 710 AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp> 740 750 AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser> 770 780 790 810 TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr> 840 850 ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys> 870 880 890 900 910 AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu> 930 940 950 GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys> 1000 970 980 990 CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp> 1030 1040 1050 CTG TGG GCT GGG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser> 1060 1070 1080 1090 GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn> 1120 1130 1140 GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp> 1160 1180 1190 1200

#### SUBSTITUTE SHEET (RULE 26)

Fig.21C.

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AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu> 1220 1230 1240 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro> 1270 1280 1250 1260 1290 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg> 1320 1330 1340 GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC TGG AGT GAG Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Trp Ser Glu> 1390 1360 1370 1380 TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu> 1420 1430 1410 CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 1460 1470 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> 1500 1510 1520 ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp> 1560 1570 1540 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly> 1610 1620 1600 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn> 1660 1650 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp> 1700 1710 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> 1730 1750 1760 1740 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

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### Fig.21D.

1780 1790 1800 1810 1820 CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn> 1830 1840 1850 1860 1870 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> 1900 1890 1910 1920 * GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr> 1940 1950 ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys> 1970 1980 1990 2000 CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys> 2020 2030 2040 2050 2060 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu> 2070 2080 TCC CTG TCT CCG GGT AAA TGA Ser Leu Ser Pro Gly Lys ***>

20/63 20 30 ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu> 50 60 70 80 CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG Pro Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly> 100 110 120 130 AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp> 160 170 180 TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val> 200 210 220 230 TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro> 250 260 270 280 CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn> 300 310 320 GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr> 370 340 350 360 TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe> 390 400 410 420 430 GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Gln Ala Thr Gln> 450 460 470 ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu> 500 510 ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>

#### SUBSTITUTE SHEET (RULE 26)

AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>

560

550

530

21/63 Fig.22B. 590 600 610 TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe> 640 650 660 TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg> 690 710 700 AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp> 740 750 * * 760 AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GGG AAC Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Gly Asn> 770 780 790 800 ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC ATG AGC ATC Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr Met Ser Ile> 850 * * 840 830 TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC AGC ACC GAG Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys Ser Thr Glu> 870 890 900 880 CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA GCC CAC ACG Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu Ala His Thr> 930 940 TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC CAC CTG CTC Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys His Leu Leu> 980 990 ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC CTG TGG GCT Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp Leu Trp Ala> 1040 1030 GGG CAG CAG CTG TGG AAG GGC TCC TTC AAG CCC AGC GAG CAT GTG Gly Gln Gln Leu Trp Lys Gly Ser Phe Lys Pro Ser Glu His Val> 1090 1080 1060 AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT GTC TCC GAC Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn Val Ser Asp> 1130 ACT CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC AAT TAC CTG Thr Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp Asn Tyr Leu> 1160 1170 1180 1190 1200

Fig.22C. 22/63

TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA AAC GAC CCG Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu Asn Asp Pro> 1220 1230 1210 GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC TCC CTC CGC Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro Ser Leu Arg> 1270 1280 1250 1260 ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG GCA CGG GTG Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg Ala Arg Val> 1320 1310 1300 1330 AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG TGG AGC CCC Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu Trp Ser Pro> 1370 1380 1360 AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG CAG TCC GGA Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu Gln Ser Glv> 1410 1420 1430 1400 GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly> 50 1460 1470 GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met> 1500 1510 1520 ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His> 1560 1570 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val> 1600 1610 CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr> 1660 1670 1650 CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly> 1700 1710 1720 AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile> 1750 1730 1740 1760 GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val>

## Fig.22D.

1780 1790 1800 1810 TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser> 1840 1850 1830 1860 * CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu> 1880 1890 1900 1910 TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro> 1940 1950 1960 * GTG CTG GAC TCC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val> 1970 1980 1990 2000 2010 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met> 2030 2020 2040 2050 2060 CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser> 2070 CCG GGT AAA TGA Pro Gly Lys ***>

Fig.23A.

		3													
	*	1	.0	+		20			30			4	0		
ATG Met	GTG Val	AAG Lvs	CCA	TCA	TTA	CCA	TTC	ACA	TCC	CTC	TTA	TTC	CTG	CAG	CTG Leu>
		-,0		001	Dea			****	Jer	Leu	Deu	rne	beu	GIII	rea>
50 *		*	60		*	7	70 *	*		80		*	90		
CCC	CTG	CTG	GGA	GTG	GGG	CTG	AAC	ACG	ACA	ATT	CTG	ACG	CCC	AAT	GGG
Pro	Leu	Leu	Gly	Val	Gly	Leu	Asn	Thr	Thr	Ile	Leu	Thr	Pro	Asn	Gly>
10	00	*	1	L10 *		*	120		*	13	3 O *	*	1	.40	
AAT	GAA	GAC	ACC	ACA	GCT	GAT	TTC	TTC	CTG	ACC	ACT	ATG	ccc	ACT	GAC
Asn	Glu	Asp	Thr	Thr	Ala	Asp	Phe	Phe	Leu	Thr	Thr	Met	Pro	Thr	Asp>
*	150		*	16	50 *	*	1	.70 ★		*	180		*	19	0
TCC	CTC	AGT	GTT	TCC	ACT	CTG	CCC	CTC	CCA	GAG	GTT	CAG	TGT	$\mathbf{T}\mathbf{T}\mathbf{T}$	GTG
Ser	Leu	Ser	Val	Ser	Thr	Leu	Pro	Leu	Pro	Glu	Val	Gln	Cys	Phe	Val>
*	2	* 00		*	210		*	22	20	*	2	30		*	240
TTC	AAT	GTC	GAG	TAC	ATG	AAT	TGC	ACT	TGG	AAC	AGC	AGC	TCT	GAG	CCC
Phe	Asn	Val	Glu	Tyr	Met	Asn	Cys	Thr	Trp	Asn	Ser	Ser	Ser	Glu	Pro>
	*	25	50 *	*	2	260 *		*	270		*	28	30 *	*	
CAG	CCT	ACC	AAC	CTC	ACT	CTG	CAT	TAT	TGG	TAC	AAG	AAC	TCG	GAT	AAT
GIII	PIO	TILL	ASI	rea	THE	Leu	HIS	TYI	1.LD	IXI	ьys	Asn	Ser	Asp	Asn>
290		*	300		*	31	LO *	*	3	320		*	330		*
290 * GAT	AAA	* GTC	300 * CAG	AAG	* TGC	AGC	* CAC	* TAT	СТА	* TTC	TCT	* GAA	* GAA	ATC	* ACT
290 * GAT	AAA	* GTC	300 * CAG	AAG Lys	* TGC Cys	AGC	* CAC	* TAT Tyr	СТА	* TTC	TCT Ser	* GAA Glu	* GAA	ATC Ile	* ACT Thr>
290 * GAT	AAA Lys	* GTC	300 * CAG Gln	AAG Lys 350	* TGC Cys	AGC	* CAC	* TAT Tyr	СТА	* TTC	Ser	* GAA Glu	* GAA Glu	ATC Ile	* ACT Thr>
290 * GAT Asp 34	AAA Lys 10 * GGC	* GTC Val	300 CAG Gln	Lys 350 * TTG	Cys	AGC Ser	CAC His 360	Tyr GAG	CTA Leu * ATC	TTC Phe 37	Ser	Glu + TAC	GAA Glu CAA	Ile 880 * ACA	Thr>
290 * GAT Asp 34	AAA Lys 10 * GGC	* GTC Val	300 CAG Gln	Lys 350 * TTG	Cys	AGC Ser	CAC His 360	Tyr GAG	CTA Leu * ATC	TTC Phe 37	Ser	Glu + TAC	GAA Glu CAA	Ile 880 * ACA	Thr>
290  GAT Asp  34  TCT Ser	AAA Lys 10 * GGC Gly 390	* GTC Val  * TGT Cys	CAG Gln CAG Gln	Lys 350 * TTG Leu 40	CAA Gln 00	AGC Ser * AAA Lys	CAC His 360 * AAG Lys	GAG Glu 110	CTA Leu * ATC Ile	TTC Phe 37 CAC His	Ser 70 * CTC Leu 420 *	Glu * TAC Tyr	GAA Glu CAA Gln	Ile 880 * ACA Thr	Thr> TTT Phe>
290  * GAT Asp  34 TCT Ser  * GTT	AAA Lys 10 * GGC Gly 390 *	* GTC Val  * TGT Cys	CAG Gln CAG Gln	Lys 350 * TTG Leu 40 CAG	Cys CAA Gln 00 * GAC	AGC Ser * AAA Lys	CAC His 360 *AAG Lys CGG	GAG Glu 110 *	CTA Leu * ATC Ile	TTC Phe 37 CAC His	Ser 70 * CTC Leu 420 * AGA	Glu * TAC Tyr	GAA Glu CAA Gln	Ile 380 ACA Thr 4:	Thr> TTT Phe> 30 * CAG
290  * GAT Asp  34 TCT Ser  * GTT	AAA Lys GGC Gly 390 * GTT Val	* GTC Val  * TGT Cys CAG Gln	CAG Gln CAG Gln	Lys 350 * TTG Leu 40 CAG	Cys CAA Gln 00 * GAC	AGC Ser * AAA Lys	CAC His 360 *AAG Lys CGG	GAG Glu 110 *	CTA Leu * ATC Ile	TTC Phe 37 CAC His	Ser 70 * CTC Leu 420 * AGA	Glu * TAC Tyr	GAA Glu CAA Gln	Ile 380 ACA Thr 4:	Thr> TTT Phe>
290  * GAT Asp  34 TCT Ser  * GTT	AAA Lys GGC Gly 390 * GTT Val	* GTC Val  * TGT Cys	CAG Gln CAG Gln	Lys 350 * TTG Leu 40 CAG	Cys CAA Gln 00 * GAC	AGC Ser * AAA Lys	CAC His 360 *AAG Lys CGG	GAG Glu 110 * GAA Glu	CTA Leu * ATC Ile	TTC Phe 37 CAC His	Ser  O  CTC Leu  420  AGA Arg	Glu * TAC Tyr	GAA Glu CAA Gln	Ile 380 ACA Thr 4:	Thr> TTT Phe> 30 * CAG
290  * GAT Asp  34 TCT Ser  * GTT Val	AAA Lys 10 * GGC Gly 390 * GTT Val	* GTC Val  * TGT Cys  CAG Gln 440 * AAA	CAG Gln  CAG Gln  CTC  CTC	Lys 50 TTG Leu 40 CAG Gln * CAG	CAA Gln OO * GAC Asp 450 *	AGC Ser * AAA Lys * CCA Pro	CAC His 360 * AAG Lys CGG Arg	GAG Glu 110 GAA Glu ATC	CTA Leu * ATC Ile CCC Pro	TTC Phe 37 CAC His * AGG Arg	Ser  O  CTC Leu  420  * AGA Arg	TAC Tyr CAG Gln	GAA Glu CAA Gln  * GCC Ala	ACA Thr ACA Thr ACA AACA	Thr> TTT Phe> CAG Gln> 480 *
290  * GAT Asp  34 TCT Ser  * GTT Val	AAA Lys 10 * GGC Gly 390 * GTT Val	* GTC Val  * TGT Cys CAG Gln 40 * AAA Lys	CAG Gln  CAG Gln  CTC  Leu  CTG  Leu	Lys 50 TTG Leu 40 CAG Gln * CAG	CAA Gln O0 * GAC Asp 450 *	AGC Ser * AAA Lys * CCA Pro	CAC His 360 * AAG Lys CGG Arg	GAG Glu 110 GAA Glu ATC	CTA Leu * ATC Ile CCC Pro	TTC Phe 37 CAC His * AGG Arg	Ser  O  CTC Leu  420  * AGA Arg	TAC TYr CAG Gln 470 * CCA Pro	GAA Glu  CAA Gln  * GCC Ala  GAG Glu	ACA Thr ACA Thr ACA AACA	Thr> TTT Phe> CAG Gln> 480
290  GAT Asp  34  TCT Ser  * GTT Val  ATG Met	AAA Lys 10 * GGC Gly 390 * GTT Val	* GTC Val  * TGT Cys  CAG Gln 440 * AAA Lys	CAG Gln  CAG Gln  CTC Leu  CTG Leu	Lys 50 * TTG Leu 40 CAG Gln * CAG Gln	CAA Gln 00 * GAC Asp 450 * AAT	AGC Ser * AAA Lys * CCA Pro CTG Leu	CAC His 360 * AAG Lys CGG Arg * GTG Val	GAG Glu 110 GAA Glu ATC Ile	CTA Leu * ATC Ile CCC Pro 60 * CCC Pro	TTC Phe 37 CAC His * AGG Arg TGG Trp	Ser  O  CTC Leu  420  AGA Arg  GCT Ala	TAC TYT CAG Gln 470 * CCA Pro	GAA Glu CAA Gln GCC Ala GAG Glu	ACA Thr 4: ACA Thr ACA ACA Thr	Thr> TTT Phe> CAG Gln> 480 * CTA Leu>
290  GAT Asp  34  TCT Ser  * GTT Val  ATG Met	AAA Lys 10 * GGC Gly 390 * GTT Val CTA Leu	CAG Gln AAA Lys	CAG Gln CAG Gln CTC Leu CTG Leu	Lys 50 * TTG Leu 40 CAG Gln * CAG Gln * CAG	CAA Gln 00 * GAC Asp 450 * AAT Asn	AGC Ser * AAA Lys * CCA Pro CTG Leu 500 * GAA	CAC His 360 * AAG Lys CGG Arg * GTG Val	GAG Glu 110 GAA Glu ATC Ile	CTA Leu * ATC Ile CCC Pro 50 * CCC Pro	TTC Phe 37 CAC His AGG Arg TGG Trp	Ser  O  CTC Leu  420  AGA Arg  GCT Ala	TAC Tyr CAG Gln 470 * CCA Pro	GAA Glu CAA Gln * GCC Ala GAG Glu 20	ACA Thr ACA Thr ACA AAC AAC	Thr> TTT Phe> CAG Gln> 480 * CTA
290  GAT Asp  34  TCT Ser  * GTT Val  ATG Met	AAA Lys 10 * GGC Gly 390 * GTT Val CTA Leu	CAG Gln AAA Lys	CAG Gln  CAG Gln  CTC Leu  CTG Leu  AAA Lys	Lys 50 * TTG Leu 40 CAG Gln * CAG Gln * CAG	CAA Gln 00 * GAC Asp 450 * AAT Asn	AGC Ser * AAA Lys * CCA Pro CTG Leu 500 * GAA Glu	CAC His 360 * AAG Lys CGG Arg * GTG Val	GAG Glu 110 GAA Glu ATC Ile	CTA Leu  ATC Ile  CCC Pro  510  * CTA Leu	TTC Phe 37 CAC His AGG Arg TGG Trp GAA Glu	Ser  O  CTC Leu  420  AGA Arg  GCT Ala	TAC Tyr CAG Gln 470 * CCA Pro	GAA Glu CAA Gln GCC Ala GAG Glu 20 TGG Trp	ACA Thr ACA Thr ACA AAC AAC	Thr> TTT Phe> CAG Gln> 480 * CTA Leu>
290  GAT Asp  34  TCT Ser  * GTT Val  ATG Met  ACA Thr  530 *	AAA Lys 40 * GGC Gly 390 GTT Val CTA Leu	* GTC Val  * TGT Cys CAG Gln 40 * AAA Lys CAC His	CAG Gln CAG Gln CTC Leu CTG Leu CTG Leu S0 * AAA Lys 540	Lys 50 * TTG Leu 40 CAG Gln * CAG Gln CTG Leu	Cys CAA Gln 00 * GAC Asp 450 * AAT Asn AGT Ser	AGC Ser * AAA Lys * CCA Pro CTG Leu 500 * GAA Glu	CAC His 360 * AAG Lys CGG Arg * GTG Val TCC Ser 50 *	GAG Glu GAA Glu ATC Ile CAG Gln	CTA Leu  ATC Ile  CCC Pro  510  CTA Leu	TTC Phe 37 CAC His AGG Arg TGG Trp GAA Glu 560	Ser  O  CTC Leu  420  AGA Arg  GCT Ala  * CTG Leu	TAC Tyr CAG Gln 470 * CCA Pro 5 AAC Asn	GAA Glu CAA Gln  * GCC Ala GAG Glu 20 * TGG Trp 570	ACA Thr ACA Thr ACA ASN AAC ASN	Thr> TTT Phe> 30 * CAG Gln> 480 * CTA Leu> AAC Asn>
290  GAT Asp  34  TCT Ser  * GTT Val  ATG Met  ACA Thr  530  AGA	AAA Lys  10 * GGC Gly 390 GTT Val CTA Leu  * CTT Leu	* GTC Val  * TGT Cys CAG Gln 40 * AAA Lys CAC His	CAG Gln CAG Gln CTC Leu CTG Leu CTG Leu AAA Lys AAC	Lys 50 * TTG Leu 40 CAG Gln * CAG Gln Leu CTG Leu	Cys CAA Gln 00 * GAC Asp 450 * AAT Asn AGT Ser	AGC Ser  * AAA Lys  * CCA Pro  CTG Leu  500  * GAA Glu  55	CAC His 360 * AAG Lys CGG Arg * GTG Val TCC Ser 60 * GAG	GAG Glu GAA Glu ATC Ile CAG Gln	CTA Leu  ATC Ile  CCC Pro  510 CTA Leu  TTG	TTC Phe 37 CAC His AGG Arg TGG Trp GAA Glu GTG	Ser  O  CTC Leu  420  AGA Arg  GCT Ala  * CTG Leu  CAG	TAC TYr CAG Gln 470 CCA Pro 5 AAC Asn	GAA Glu CAA Gln  * GCC Ala GAG Glu 20 * TGG Trp 570 * CGG	ACA Thr ACA Thr ACA ASN AAC ASN	Thr> TTT Phe> CAG Gln> 480 * CTA Leu>

25/63 580 600 610 TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe> 640 650 660 TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg> 690 700 710 720 AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp> 750 AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser> 770 780 790 800 TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr> 830 840 850 ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys> 870 880 890 900 AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu> 930 940 GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys> 970 980 990 1000 CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp> 1020 1030 CTG TGG GCT GGG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser> 1060 1070 1080 1090 GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn> 1130 1140 GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp> 1160 1170 1180 1190 1200

### **SUBSTITUTE SHEET (RULE 26)**

Fig.23C.

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AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu> 1220 1230 AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro> 1270 1280 TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg> 1310 1320 1330 GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr Asn Thr Trp Ser Glu> 1370 1360 1380 TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu> 1430 1400 1410 1420 CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 1450 1460 1470 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> 1500 1510 1490 1520 * ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp> 1550 1560 1570 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly> 1600 1610 1620 GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn> 1650 1660 1670 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp> 1700 * * * 1710 1690 1720 CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro> 1760 1740 1750 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

## Fig.23D.

1780 1790 1800 1810 1820 CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn> 1830 1840 1850 1860 1870 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile> 1890 1900 1910 GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr> 1940 1960 1950 ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys> 1970 1980 1990 2000 CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys> 2020 2030 2040 2050 2060 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu> 2070 2080 TCC CTG TCT CCG GGT AAA TGA Ser Leu Ser Pro Gly Lys ***>

Fig.24A.

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30 20 Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro> 50 70 80 GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg> 110 120 130 GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro> 170 * 160 180 GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys> 200 210 220 230 CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg> **270** 250 260 CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys> 300 310 320 TAC CGG GCC GGC CGA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val> 360 340 370 350 CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser> 400 410 420 AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr> 470 450 460 AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp> 490 500 510 TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys> 550 560 CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

29/63 610 620 * * * * 600 TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe> 640 650 660 CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val> 690 700 710 ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp> 30 740 750 760 CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg> TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp> 820 830 840 850 860 820 CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His> * * * * * * * * GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser> 920 930 940 950 960 GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser> 980 990 * * CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG ACC GGT GGC GCG CCT Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Thr Gly Gly Ala Pro> 1020 1030 1040 TCA GGT GCT CAG CTG GAA CTT CTA GAC CCA TGT GGT TAT ATC AGT CCT Ser Gly Ala Gln Leu Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro> 1070 1080 1060 GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val> 1120 1130 CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT TAC ATT Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile> 1200 1160 1170 1190 1180

## Fig.24C. 30/63

GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT ACT ATC Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile> 1220 1210 1230 1240 * ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT TCA TTA Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu> 1250 1260 1270 1280 AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT GAA CAG Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln> 1310 1300 1330 1320 AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro> 1370 1360 1380 AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu> 1410 1420 * TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys> 1460 1470 1480 TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA CGT GAC Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp> 1510 * 1490 1520 1500 ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT GTC AAC Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn> 1550 1560 1570 ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT ACA TCA Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser> 1620 1600 1610 GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT CCG CCA Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro> 1650 1660 1670 CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys> 1710 1700 TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr> 1750 1730 1740 1760 1770 AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT

### SUBSTITUTE SHEET (RULE 26)

Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro>

31/63 Fig.24D. CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA GAC CTT Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp Leu> AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT ATG AAG GAA GAT Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu Asp> GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA GAA GCA AGT GGG ATC ACC Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile Thr> TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT TTC TGG TAT AAA ATA GAT Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile Asp> CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA CAA CTC GTG TGG AAG ACA Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys Thr> TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA GTG ACT Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val Thr> CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT GCC ACA Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala Thr> AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC CTA ACA Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu Thr> GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT TTA ACT ATC CCT Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile Pro> GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA ATG GAT CTT AAA GCA TTC Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala Phe> CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG ACT ACT CCA AGG GAA TCT Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu Ser> GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG TTA TCA GAT AAA GCA CCC Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala Pro> 

#### SUBSTITUTE SHEET (RULE 26)

32/63 TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr> 2430 2420 TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val Thr> 2460 2470 2480 2490 CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG GCA TAC Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala Tyr> 2520 2530 CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA AAA AAA Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys Lys> 2560 2570 2580 GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT GTT GAT Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val Asp> 2630 2610 2620 GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA ACC ATC Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr Ile> 2660 * * 2670 2650 ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu Tyr> 2720 2710 2700 2730 ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala> 2770 2750 2760 GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr> 2810 2820 2800 ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA TCC GGG GGC GAC AAA ACT Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ser Gly Gly Asp Lys Thr> 2860 2870 2850 2840 CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser> 2890 2900 2910 GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg> 2960 2930 2950 2940 2970 ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT

Fig.24F. 33/63

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro> 2990 3000 3010 GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala> 3040 3060 3050 * AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val> 3100 * * 3080 3090 3110 AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr> 3140 3150 AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr> 3170 3190 3180 3200 ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu> 3230 3240 3260 CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys> 3280 3290 3300 CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser> 3340 3350 3330 AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp> 3380 3390 TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser> 3410 3430 3420 3440 AGG TGG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala> 3470 3480 3490 CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys> TGA

#### SUBSTITUTE SHEET (RULE 26)

34/63 Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro> GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg> GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro> GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys> CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg> CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys> TAC CGG GCC GGC CGA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val> CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser> AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr> AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp> TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys> 

### SUBSTITUTE SHEET (RULE 26)

CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>

35/63 590 610 * * 600 TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe> 660 650 * * 630 CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val> 690 700 * ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp> 730 740 750 760 CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg> 780 790 800 810 TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp> **840** 820 830 CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His> 880 890 900 GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC Val Val Gln Leu Arg Ala Gln Glu Glu Phe Gly Gln Gly Glu Trp Ser> 940 930 950 GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCG CGA TCG Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser> 980 990 CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG GAA CTT CTA GAC CCA Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Glu Leu Leu Asp Pro> 1040 1020 1030 TGT GGT TAT ATC AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn> 1060 1070 1080 1090 TTC ACT GCA GTT TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT Phe Thr Ala Val Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His> 1140 1120 1130 GTA AAT GCT AAT TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT Val Asn Ala Asn Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro> 1170 1160 1190 1180 1200

36/63 Fig.25C. AAG GAG CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe> 1220 1210 1230 1240 ACA GAT ATA GCT TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr> 1260 1270 1280 1290 TTC GGA CAG CTT GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly> 1330 1300 1310 1320 1340 TTG CCT CCA GAA AAA CCT AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly> 1360 1370 1380 AAG AAA ATG AGG TGT GAG TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG Lys Lys Met Arg Cys Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu> 1410 1420 1430 1440 ACA AAC TTC ACT TTA AAA TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp> 1460 1470 TGC AAA GCA AAA CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser> 1490 1500 1510 1530 ACT GTG TAT TTT GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC Thr Val Tyr Phe Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala> 1570 1560 CTT GGG AAG GTT ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA Leu Gly Lys Val Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys> 1590 1600 1610 1620 1630 GTG AAG CCC AAT CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA Val Lys Pro Asn Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu> 1650 1660 CTG TCT AGT ATC TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT Leu Ser Ser Ile Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser> 1700 GTT ATA ATA CTA AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser> 1740 1750 1760

ACT TGG AGC CAG ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser>

37/63 Fig.25D. TTC ACT GTC CAA GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT Phe Thr Val Gln Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile> CGC TGT ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu> GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser> TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val> CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC Gln Leu Val Trp Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile> TTG GAT TAT GAA GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT Leu Asp Tyr Glu Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn> TAC ACA GTT AAT GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC Tyr Thr Val Asn Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg> TAT CTA GCA ACC CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA Tyr Leu Ala Thr Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala> GCT GTT TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA Ala Val Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val> ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp> ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val> TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp Trp Gln Glu Asp Gly> 

38/63 ACC GTG CAT CGC ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC Thr Val His Arg Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys> 2430 2410 2420 TAT TTG ATA ACA GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT Tyr Leu Ile Thr Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro> 2480 2470 2450 2460 2490 GAA TCC ATA AAG GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT Glu Ser Ile Lys Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro> 2530 2510 2520 ACT GTT CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG Thr Val Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp> 2550 2560 2570 * * 2580 GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr> 2610 2620 2630 2640 ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp> 2660 * * 2670 * TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG Ser Ser His Thr Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu> 2690 2700 2710 2720 TAC ATG GTA CGA ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT Tyr Met Val Arg Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly> 2760 2750 2770 CCA GAA TTC ACT TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA Pro Glu Phe Thr Phe Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu> 2800 2810 2820 TCC GGG GGC GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA Ser Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu> 2860 2870 2850 CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp> 2900 2910 2920 ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp> 2930 2960 2940 2950 2970 GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC

Fig.25F.

Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly>
298	30 *	*	29	90		· •	000		*	301	LO *	*	30	20	
GTG Val	GAG Glu	GTG Val	CAT His	AAT Asn	GCC Ala	AAG Lys	ACA Thr	AAG Lys	CCG Pro	CGG Arg	GAG Glu	GAG Glu	CAG Gln	TAC Tyr	AAC Asn>
*	3030		*	304	10	*	3 (	50		3	3060			307	0
AGC Ser	ACG Thr	TAC Tyr	CGT Arg	GTG Val	GTC Val	AGC Ser	GTC Val	CTC Leu	ACC Thr	GTC Val	CTG Leu	CAC His	CAG Gln	GAC Asp	TGG Trp>
		080			090			310				10			120
CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	* GTC	TCC	AAC	* AAA	GCC	* CTC	* CCA
Leu	Asn			Glu	Tyr	Lys	Суѕ	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro>
	*	313	30 *	*	31	.40 *		*	3150		*	316	50 *	*	
			GAG Glu												GAA Glu>
3170			3180	-10		319		2,5		200	Cly			ALG	Gruz
*		* :	*		*		*	*		*		*	3210		*
CCA Pro	CAG Gln	GTG Val	TAC Tyr	ACC Thr	CTG Leu	CCC Pro	CCA Pro	TCC Ser	CGG Arg	GAT Asp	GAG Glu	CTG Leu	ACC Thr	AAG Lys	AAC Asn>
322	20 *	*	32	230		*	3240		*	325	50 *	*	32	260	
CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC
		ser	Leu			rea			GIA			Pro	ser	Asp	Ile>
*	3270		*	328	*	*	32	290 *		*	3300		*	331	LO *
GCC Ala	GTG Val	GAG Glu	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC Thr>
							013			014			LYL		
*		320		*	3330		*	334	*	*		350 *		*	3360
ACG Thr	CCT Pro	CCC Pro	GTG Val	CTG Leu	GAC Asp	TCC Ser	GAC Asp	GGC Gly	TCC Ser	TTC Phe	TTC Phe	CTC Leu	TAC Tvr	AGC Ser	AAG Lys>
		33′				380	_		3390			34			
	*		*	*		*		*	*		*		*	*	
CTC Leu	ACC	GTG Val	GAC Asp	AAG Lys	AGC Ser	AGG Arg	TGG Trp	CAG Gln	CAG Gln	GGG Gly	AAC Asn	GTC Val	TTC Phe	TCA Ser	TGC Cys>
3410			3420			343	30		34	440			3450		
*	cmc.	*	*		*		*	*		*		*	*		*
Ser	Val	ATG Met	His	GAG Glu	GCT Ala	CTG Leu	CAC His	AAC Asn	CAC His	TAC Tyr	ACG Thr	CAG Gln	AAG Lys	AGC Ser	CTC Leu>
340				470											
maa	*	*	000	*		*									
			CCG Pro												

40/63 Fig.26A. ATG GTG CTT CTG TGG TGT GTA GTG AGT CTC TAC TTT TAT GGA ATC CTG Met Val Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu> CAA AGT GAT GCC TCA GAA CGC TGC GAT GAC TGG GGA CTA GAC ACC ATG Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met> AGG CAA ATC CAA GTG TTT GAA GAT GAG CCA GCT CGC ATC AAG TGC CCA Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro> CTC TTT GAA CAC TTC TTG AAA TTC AAC TAC AGC ACA GCC CAT TCA GCT Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala> GGC CTT ACT CTG ATC TGG TAT TGG ACT AGG CAG GAC CGG GAC CTT GAG Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu> GAG CCA ATT AAC TTC CGC CTC CCC GAG AAC CGC ATT AGT AAG GAG AAA Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys> GAT GTG CTG TGG TTC CGG CCC ACT CTC CTC AAT GAC ACT GGC AAC TAT Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr> ACC TGC ATG TTA AGG AAC ACT ACA TAT TGC AGC AAA GTT GCA TTT CCC Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro> TTG GAA GTT GTT CAA AAA GAC AGC TGT TTC AAT TCC CCC ATG AAA CTC Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu> CCA GTG CAT AAA CTG TAT ATA GAA TAT GGC ATT CAG AGG ATC ACT TGT Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys> CCA AAT GTA GAT GGA TAT TTT CCT TCC AGT GTC AAA CCG ACT ATC ACT Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr> TGG TAT ATG GGC TGT TAT AAA ATA CAG AAT TTT AAT AAT GTA ATA CCC Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro>

### SUBSTITUTE SHEET (RULE 26)

41/63 590 600 610 GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT GGA Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly> 660 630 650 AAT TAC ACA TGT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT CAT Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His> 690 700 710 720 CTC ACC AGG ACT CTG ACT GTA AAG GTA GGC TCT CCA AAA AAT GCA Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala> 740 750 760 GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG AAA Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys> 770 790 800 810 GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT TTT Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe> 830 840 850 CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA AAA Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys> 880 890 900 CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT CAT Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His> 940 AGT AGA ACA GAA GAT GAA ACA AGA ACT CAG ATT TTG AGC ATC AAG AAA Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys> 980 990 GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA AGT Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser> 1030 1040 GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GTG CCA Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro> 1060 1070 1080 1090 GCT CCA AGA TAC ACA GTG TCC GGT GGC GCG CCT ATG CTG AGC GAG GCT Ala Pro Arg Tyr Thr Val Ser Gly Gly Ala Pro Met Leu Ser Glu Ala> 1130 1150 GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA ATT TTA GTG TCA TCT GCA Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val Ser Ser Ala> 1160 1170 1180 1190 1200

### SUBSTITUTE SHEET (RULE 26)

#### 42/63 Fig.26C. AAT GAA ATT GAT GTT CGT CCC TGT CCT CTT AAC CCA AAT GAA CAC AAA Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn Glu His Lys> 1220 1210 1230 GGC ACT ATA ACT TGG TAT AAG GAT GAC AGC AAG ACA CCT GTA TCT ACA Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro Val Ser Thr> 1250 1260 1270 1280 GAA CAA GCC TCC AGG ATT CAT CAA CAC AAA GAG AAA CTT TGG TTT GTT Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu Trp Phe Val> 1300 1330 1310 1320 1340 CCT GCT AAG GTG GAG GAT TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val Val Arg Asn> 1360 1370 1380 TCA TCT TAC TGC CTC AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn> 1410 1420 1430 GAG CCT AAC TTA TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu> 1450 1460 1470 1480 CCC GTT GCA GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr Met Glu Phe Phe> 1490 1500 1510 1520 1530 AAA AAT GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys> 1550 1560 1570 AAA CCT CTA CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG Lys Pro Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg> 1600 1620 1610 CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT TGT Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr Cys> 1650 1660

His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr Arg Val> 1700 1710 1690 ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG CCT GTG ATT Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg Pro Val Ile> 1740 1750 1760 1770 GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG GGA TCC CAG ATA

CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC CGG GTA

1670

1680

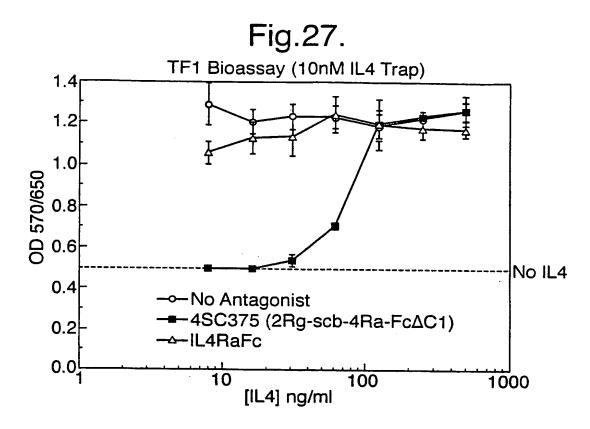
### SUBSTITUTE SHEET (RULE 26)

Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly Ser Gln Ile>

43/63 Fig.26D. CAA TTG ATC TGT AAT GTC ACC GGC CAG TTG AGT GAC ATT GCT TAC TGG Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp Ile Ala Tyr Trp> AAG TGG AAT GGG TCA GTA ATT GAT GAA GAT GAC CCA GTG CTA GGG GAA Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro Val Leu Gly Glu> GAC TAT TAC AGT GTG GAA AAT CCT GCA AAC AAA AGA AGG AGT ACC CTC Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg Arg Ser Thr Leu> ATC ACA GTG CTT AAT ATA TCG GAA ATT GAG AGT AGA TTT TAT AAA CAT Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg Phe Tyr Lys His> CCA TTT ACC TGT TTT GCC AAG AAT ACA CAT GGT ATA GAT GCA GCA TAT Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile Asp Ala Ala Tyr> ATC CAG TTA ATA TAT CCA GTC ACT AAT TCC GGA GAC AAA ACT CAC ACA Ile Gln Leu Ile Tyr Pro Val Thr Asn Ser Gly Asp Lys Thr His Thr> TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe> CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro> GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val> AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr> AAG CCG CGG GAG GAG CAG TAC AGC ACG TAC CGT GTG GTC AGC GTC Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val> CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

## Fig.26E.

AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser> 2410 2420 2430 2440 AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro> 2450 2470 2460 2480 2490 TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val> 2500 2510 2520 2530 2540 AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly> 2560 2570 2580 CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp> 2600 2610 2620 2630 GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp> 2650 2660 2670 2680 CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His> 2690 2700 2710 2720 2730 AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>



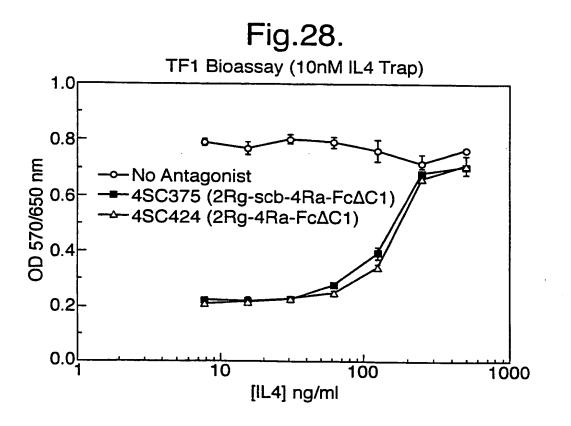


Fig.29.

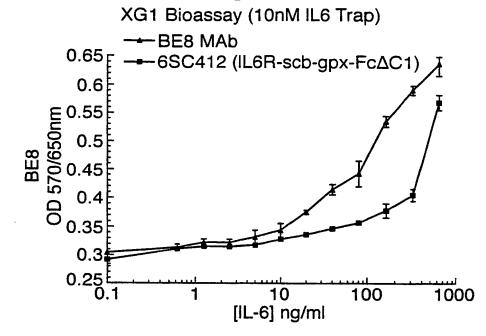
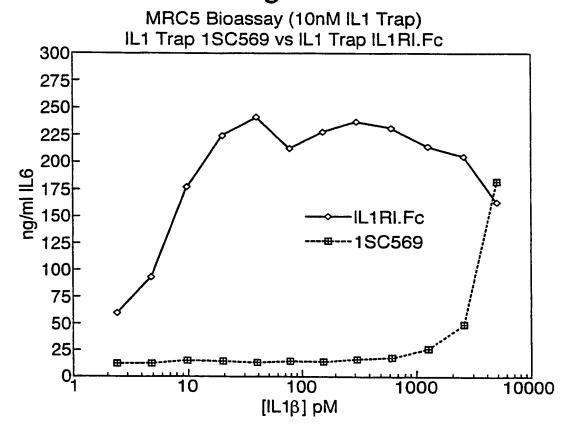


Fig.30.



# Fig.31A.

			10			20			30			4	10		
	*		*	*		*		*	*		*		*	*	
ATG	GTG	TGG	CTT	TGC	TCT	GGG	CTC	CTG	TTC	CCT	GTG	AGC	TGC	CTG	GTC
TAC	CAC	ACC	GAA	ACG	AGA	CCC	GAG	GAC	AAG	GGA	CAC	TCG	ACG	GAC	CAG
Met	Val	Trp	Leu	Cys	Ser	Gly	Leu	Leu	Phe	Pro	Val	Ser	Суѕ	Leu	Val>
50			60			•	70			80			90		
*		*	*		*		*	*		*		*	*		*
CTG	CTG	CAG	GTG	GCA	AGC	TCT	GGG	AAC	ATG	AAG	GTC	TTG	CAG	GAG	CCC
GAC	GAC	GTC	CAC	CGT	TCG	AGA	CCC	TTG	TAC	TTC	CAG	AAC	GTC	CTC	GGG
Leu	ren	Gin	Val	Ala	Ser	Ser	Gly	Asn	Met	Lys	Val	Leu	Gln	Glu	Pro>
10	00		1	110			120			1:	30		1	L <b>4</b> 0	
	*	*		*		*	*		*		*	*		*	
ACC	TGC	GTC	TCC	GAC	TAC	ATG	AGC	ATC	TCT	ACT	TGC	GAG	TGG	AAG	ATG
TGG	ACG	CAG	AGG	CTG	ATG	TAC	TCG	TAG	AGA	TGA	ACG	CTC	ACC	TTC	TAC
Thr	Cys	Val	Ser	Asp	Tyr	Met	Ser	Ile	Ser	Thr	Cys	Glu	Trp	Lys	Met>
	150			16	50			L70			180			10	90
*	*		*		*	*		*		*	*		*	_,	*
TAA	GGT	CCC	ACC	AAT	TGC	AGC	ACC	GAG	CTC	CGC	CTG	TTG	TAC	CAG	CTG
TTA	CCA	GGG	TGG	TTA	ACG	TCG	TGG	CTC	GAG	GCG	GAC	AAC	ATG	GTC	GAC
Asn	Gly	Pro	Thr	Asn	Cys	Ser	Thr	Glu	Leu	Arg	Leu	Leu	Tyr	Gln	Leu>
	2	200			210			22	20		. :	230			240
*		*		*	*		*		*	*		230		*	240
* GTT	TTT	* CTG	CTC	* TCC	* GAA	GCC	CAC	ACG	* TGT	ATC	ССТ	* GAG	AAC	* AAC	*
CAA	TTT AAA	* CTG GAC	GAG	AGG	* GAA CTT	GCC CGG	CAC GTG	ACG TGC	* TGT ACA	ATC TAG	CCT GGA	* GAG CTC	TTG	TTG	GGA
CAA	TTT AAA	* CTG GAC	GAG	AGG	* GAA CTT	CGG	CAC GTG	ACG TGC	* TGT ACA	ATC TAG	CCT GGA	* GAG CTC	TTG	TTG	*
CAA	TTT AAA	* CTG GAC	GAG Leu	AGG	* GAA CTT Glu	CGG	CAC GTG	ACG TGC	* TGT ACA	ATC TAG	CCT GGA	* GAG CTC	TTG Asn	TTG	* GGA
Val	TTT AAA Phe	* CTG GAC Leu	GAG Leu 50	AGG Ser	GAA CTT Glu	CGG Ala 260	CAC GTG His	ACG TGC Thr	* TGT ACA Cys 270 *	ATC TAG Ile	CCT GGA Pro	* GAG CTC Glu	TTG Asn 30	TTG Asn	* GGA CCT Gly>
CAA Val GGC	TTT AAA Phe *	* CTG GAC Leu 25	GAG Leu 50 *	AGG Ser * GTG	GAA CTT Glu TGC	CGG Ala 260 *	CAC GTG His	ACG TGC Thr	* TGT ACA Cys 270 * ATG	ATC TAG Ile	CCT GGA Pro *	GAG CTC Glu 28	TTG Asn 80 *	TTG Asn *	* GGA CCT Gly>
CAA Val GGC CCG	TTT AAA Phe  * GCG CGC	CTG GAC Leu 25 GGG CCC	GAG Leu 50 * TGC ACG	AGG Ser * GTG CAC	GAA CTT Glu TGC ACG	CGG Ala 260 * CAC GTG	CAC GTG His	ACG TGC Thr * CTC GAG	TGT ACA Cys 270 * ATG TAC	ATC TAG Ile GAT CTA	CCT GGA Pro * GAC CTG	GTG	TTG Asn 30 * GTC CAG	TTG Asn * AGT TCA	* GGA CCT Gly>
CAA Val GGC CCG	TTT AAA Phe  * GCG CGC	CTG GAC Leu 25 GGG CCC	GAG Leu 50 * TGC ACG	AGG Ser * GTG CAC	GAA CTT Glu TGC ACG	CGG Ala 260 * CAC GTG	CAC GTG His	ACG TGC Thr * CTC GAG	TGT ACA Cys 270 * ATG TAC	ATC TAG Ile GAT CTA	CCT GGA Pro * GAC CTG	GTG	TTG Asn 30 * GTC CAG	TTG Asn * AGT TCA	* GGA CCT Gly>
GGC CCG Gly	TTT AAA Phe  * GCG CGC	CTG GAC Leu 25 GGG CCC	GAG Leu 50 * TGC ACG	AGG Ser * GTG CAC	GAA CTT Glu TGC ACG	CGG Ala 260 * CAC GTG	CAC GTG His CTG GAC Leu	ACG TGC Thr * CTC GAG	TGT ACA Cys 270  * ATG TAC Met	ATC TAG Ile GAT CTA	CCT GGA Pro * GAC CTG	GTG	TTG Asn 30 * GTC CAG	TTG Asn * AGT TCA	* GGA CCT Gly>
GGC CCG Gly 290	TTT AAA Phe * GCG CGC Ala	CTG GAC Leu 25 GGG CCC Gly	GAG Leu 50 * TGC ACG Cys 300	* GTG CAC Val	GAA CTT Glu TGC ACG Cys	CGG Ala 260 * CAC GTG His	CAC GTG His CTG GAC Leu	ACG TGC Thr * CTC GAG Leu	TGT ACA Cys 270 * ATG TAC Met	ATC TAG Ile GAT CTA Asp	CCT GGA Pro * GAC CTG Asp	* GAG CTC Glu 28 GTG CAC Val	TTG Asn 30 * GTC CAG Val 330	TTG Asn * AGT TCA Ser	* GGA CCT Gly> GCG CGC Ala>
GGC CCG Gly 290	TTT AAA Phe  * GCG CGC Ala	CTG GAC Leu 25 GGG CCC Gly * TAT	GAG Leu 50 * TGC ACG Cys 300 *	AGG Ser * GTG CAC Val	GAA CTT Glu TGC ACG Cys	CGG Ala 260 * CAC GTG His	CAC GTG His CTG GAC Leu	ACG TGC Thr * CTC GAG Leu	* TGT ACA Cys 270  * ATG TAC Met	ATC TAG Ile GAT CTA Asp	CCT GGA Pro * GAC CTG Asp	* GAG CTC Glu 28 GTG CAC Val	TTG Asn % GTC CAG Val 330 *	TTG Asn * AGT TCA Ser	GGA CCT Gly> GCG CGC Ala>
GGC CCG Gly 290 * GAT CTA	TTT AAA Phe  * GCG CGC Ala AAC TTG	CTG GAC Leu 25 GGG CCC Gly  TAT ATA	GAG Leu 50 * TGC ACG Cys 300 * ACA TGT	AGG Ser * GTG CAC Val	GAA CTT Glu TGC ACG Cys  * GAC CTG	CGG Ala 260 * CAC GTG His 31	CAC GTG His CTG GAC Leu TGG ACC	ACG TGC Thr * CTC GAG Leu * GCT	TGT ACA Cys 270 * ATG TAC Met	ATC TAG Ile GAT CTA Asp 320 * CAG GTC	CCT GGA Pro * GAC CTG Asp	GAG CTC Glu 28 GTG CAC Val * CTG GAC	TTG Asn  CTG CTG GAC	TTG Asn * AGT TCA Ser TGG ACC	* GGA CCT Gly> GCG CGC Ala>  * AAG TTC
GGC CCG Gly 290 * GAT CTA	TTT AAA Phe  * GCG CGC Ala AAC TTG	CTG GAC Leu 25 GGG CCC Gly  TAT ATA	GAG Leu 50 * TGC ACG Cys 300 * ACA TGT	AGG Ser * GTG CAC Val	GAA CTT Glu TGC ACG Cys  * GAC CTG	CGG Ala 260 * CAC GTG His 31	CAC GTG His CTG GAC Leu TGG ACC	ACG TGC Thr * CTC GAG Leu * GCT	TGT ACA Cys 270 * ATG TAC Met	ATC TAG Ile GAT CTA Asp 320 * CAG GTC	CCT GGA Pro * GAC CTG Asp	GAG CTC Glu 28 GTG CAC Val * CTG GAC	TTG Asn  CTG CTG GAC	TTG Asn * AGT TCA Ser TGG ACC	GGA CCT Gly> GCG CGC Ala>
GGC CCG Gly 290 * GAT CTA	TTT AAA Phe  * GCG CGC Ala AAC TTG Asn	CTG GAC Leu 25 GGG CCC Gly  TAT ATA	GAG Leu  TGC ACG Cys  ACA TGT Thr	AGG Ser * GTG CAC Val	GAA CTT Glu TGC ACG Cys	CGG Ala 260 * CAC GTG His 31	CAC GTG His CTG GAC Leu TGG ACC	ACG TGC Thr * CTC GAG Leu * GCT	TGT ACA Cys 270 * ATG TAC Met	ATC TAG Ile GAT CTA Asp 320 * CAG GTC	CCT GGA Pro * GAC CTG Asp CAG GTC Gln	GAG CTC Glu 28 GTG CAC Val * CTG GAC	TTG Asn * GTC CAG Val 330 * CTG GAC Leu	TTG Asn * AGT TCA Ser TGG ACC	* GGA CCT Gly> GCG CGC Ala>  * AAG TTC
GGC CCG Gly 290 * GAT CTA Asp	TTT AAA Phe  * GCG CGC Ala  AAC TTG Asn	CTG GAC Leu 25 GGG CCC Gly  TAT ATA Tyr	GAG Leu 50 * TGC ACG Cys 300 * ACA TGT Thr	AGG Ser * GTG CAC Val CTG GAC Leu	GAA CTT Glu TGC ACG Cys * GAC CTG Asp	CGG Ala 260 * CAC GTG His 31 CTG GAC Leu	CAC GTG His CTG GAC Leu TGG ACC Trp	ACG TGC Thr * CTC GAG Leu * GCT CGA Ala	TGT ACA Cys 270 * ATG TAC Met	GAT CTA Asp CAG GTC Gln	CCT GGA Pro * GAC CTG Asp CAG GTC GIn	* GAG CTC Glu 28 GTG CAC Val * CTG GAC Leu	TTG Asn  CTC CAG Val  CTG GAC Leu	TTG Asn  * AGT TCA Ser  TGG ACC Trp	* GGA CCT Gly> GCG CGC Ala> * AAG TTC Lys>
GGC CCG Gly 290 * GAT CTA Asp	TTT AAA Phe  * GCG CGC Ala  AAC TTG Asn 10 * TCC	CTG GAC Leu 25 GGG CCC Gly  TAT ATA Tyr  TTC	GAG Leu  TGC ACG Cys  ACA TGT Thr	* GTG CAC Val	GAA CTT Glu TGC ACG Cys  * GAC CTG Asp	CGG Ala 260 * CAC GTG His 31 CTG GAC Leu	CAC GTG His  CTG GAC Leu  TGG ACC TTP  360 * CAT	ACG TGC Thr * CTC GAG Leu * GCT CGA Ala	TGT ACA Cys 270 * ATG TAC Met GGG CCC Gly * AAA	GAT CTA Asp CAG GTC Gln	CCT GGA Pro * GAC CTG Asp CAG GTC Gln	* GAG CTC Glu 28 GTG CAC Val  * CTG GAC Leu *	TTG Asn  O  CTG CTG GAC Leu	TTG Asn  * AGT TCA Ser  TGG ACC Trp  880 *	* GGA CCT Gly> GCG CGC Ala> * AAG TTC Lys>
GGC CCG Gly 290 * GAT CTA Asp 34	TTT AAA Phe  * GCG CGC Ala  AAC TTG Asn  * TCC AGG	CTG GAC Leu 25 GGG CCC Gly * TAT ATA Tyr * TTC AAG	GAG Leu  TGC ACG Cys  ACA TGT Thr  AAG TTC	* GTG CAC Val CTG GAC Leu CCC GGG	GAA CTT Glu TGC ACG Cys  * GAC CTG Asp	CGG Ala 260 * CAC GTG His 31 CTG GAC Leu * GAG CTC	CAC GTG His  CTG GAC Leu  TGG ACC Trp  360 * CAT	ACG TGC Thr * CTC GAG Leu * GCT CGA Ala	TGT ACA Cys 270 * ATG TAC Met GGG CCC Gly * AAAA TTT	GAT CTA Asp CAG GTC GIn CCC GGG	CCT GGA Pro * GAC CTG Asp CAG GTC Gln	GAG CTC Glu 28 GTG CAC Val  * CTG GAC Leu  *	TTG Asn  O  CTG GAC Leu  CCA GGT	TTG Asn  * AGT TCA Ser  TGG ACC Trp  880 * GGA	* GGA CCT Gly> GCG CGC Ala> * AAG TTC Lys>

# Fig.31B.

	390			40	00		4	10			420			43	0
*	*		*		*	*		*		*	*		*		*
CTG	ACA	GTT	CAC	ACC	AAT	GTC	TCC	GAC	ACT	CTG	CTG	CTG	ACC	TGG	AGC
GAC	TGT	CAA	GTG	TGG	TTA	CAG	AGG	CTG	TGA	GAC	GAC	GAC	TGG	ACC	TCG
Leu	Thr	Val	His	Thr	Asn	Val	Ser	Asp	Thr	Leu	Leu	Leu	Thr	Trp	Ser>
	4	140			450			46	0		4	70			480
*		*		*	*		*		*	*		*		*	*
AAC	CCG	TAT	CCC	CCT	GAC	AAT	TAC	CTG	TAT	AAT	CAT	CTC	ACC	TAT	GCA
TTG	GGC	ATA	GGG	GGA	CTG	TTA	ATG	GAC	ATA	TTA	GTA	GAG	TGG	ATA	CGT
Asn	Pro	Tyr	Pro	Pro	Asp	Asn	Tyr	Leu	Tyr	Asn	His	Leu	Thr	Tyr	Ala>
		49	90		5	00			510			52	20		
	*		*	*		*		*	*		*		*	*	
GTC	AAC	ATT	TGG	AGT	GAA	AAC	GAC	CCG	GCA	GAT	TTC	AGA	ATC	TAT	AAC
CAG	TTG	TAA	ACC	TCA	CTT	TTG	CTG	GGC	CGT	CTA	AAG	TCT	TAG	ATA	TTG
Val	Asn	Ile	Trp	Ser	Glu	Asn	Asp	Pro	Ala	Asp	Phe	Arg	Ile	Tyr	Asn>
530			540			55	0		5	60			570		
*		*	*		*		*	*		*		*	*		*
GTG	ACC	TAC	CTA	GAA	CCC	TCC	CTC	CGC	ATC	GCA	GCC	AGC	ACC	CTG	AAG
CAC	TGG	ATG	GAT	CTT	GGG	AGG	GAG	GCG	TAG	CGT	CGG	TCG	TGG	GAC	TTC
Val	Thr	Tyr	Leu	Glu	Pro	Ser	Leu	Arg	Ile	Ala	Ala	Ser	Thr	Leu	Lys>
58	30		5	590			600			61	LO		6	520	
58	3 O *	*	5	590 *		*	600		*	61	LO *	*	(	520 *	
	*			*	AGG		*	GTG	* AGG	61 GCC	*	* GCT		*	ТАТ
TCT	* GGG	ATT	TCC	* TAC		GCA	* CGG				* TGG		CAG	* AGC	
TCT AGA	* GGG CCC	ATT TAA	TCC AGG	* TAC ATG	TCC	GCA CGT	* CGG GCC	CAC	TCC	GCC CGG	* TGG ACC	CGA	CAG GTC	* AGC TCG	
TCT AGA	* GGG CCC	ATT TAA	TCC AGG	* TAC ATG Tyr	TCC	GCA CGT	* CGG GCC Arg	CAC	TCC	GCC CGG	* TGG ACC	CGA	CAG GTC	* AGC TCG	ATA Tyr>
TCT AGA	* GGG CCC Gly	ATT TAA	TCC AGG	* TAC ATG Tyr	TCC Arg	GCA CGT	* CGG GCC Arg	CAC Val	TCC	GCC CGG	* TGG ACC Trp	CGA	CAG GTC	* AGC TCG Ser	ATA Tyr>
TCT AGA Ser	* GGG CCC Gly 630 *	ATT TAA Ile	TCC AGG Ser	* TAC ATG Tyr	TCC Arg 10	GCA CGT Ala	cgg gcc Arg	CAC Val 550	TCC Arg	GCC CGG Ala	* TGG ACC Trp 660	CGA Ala	CAG GTC Gln	* AGC TCG Ser	ATA Tyr>
TCT AGA Ser *	* GGG CCC Gly 630 * ACC	ATT TAA Ile	TCC AGG Ser * TGG	* TAC ATG Tyr 64	TCC Arg 10 * GAG	GCA CGT Ala * TGG	CGG GCC Arg	CAC Val 550 *	TCC Arg AGC	GCC CGG Ala	* TGG ACC Trp 660 * AAG	CGA Ala TGG	CAG GTC Gln *	* AGC TCG Ser 67	ATA Tyr>  O  * TCC
TCT AGA Ser * AAC TTG	GGG CCC Gly 630 * ACC	ATT TAA Ile ACC TGG	TCC AGG Ser * TGG ACC	TAC ATG Tyr 64 AGT TCA	TCC Arg 10 * GAG CTC	GCA CGT Ala * TGG ACC	* CGG GCC Arg AGC TCG	CAC Val 550 * CCC GGG	TCC Arg AGC TCG	GCC CGG Ala * ACC TGG	TGG ACC Trp 660 * AAG	CGA Ala TGG ACC	CAG GTC Gln * CAC GTG	* AGC TCG Ser 67	ATA Tyr>  O  * TCC
TCT AGA Ser * AAC TTG	GGG CCC Gly 630 * ACC TGG	ATT TAA Ile ACC TGG	TCC AGG Ser * TGG ACC	TAC ATG Tyr 64 AGT TCA	TCC Arg 10 * GAG CTC	GCA CGT Ala * TGG ACC	* CGG GCC Arg AGC TCG	CAC Val 550 * CCC GGG Pro	TCC Arg AGC TCG	GCC CGG Ala * ACC TGG	TGG ACC Trp 660 * AAG TTC Lys	CGA Ala TGG ACC	CAG GTC Gln * CAC GTG	* AGC TCG Ser 67	ATA Tyr>  O  TCC AGG
TCT AGA Ser * AAC TTG	GGG CCC Gly 630 * ACC TGG	ATT TAA Ile ACC TGG Thr	TCC AGG Ser * TGG ACC	TAC ATG Tyr 64 AGT TCA	TCC Arg 10 * GAG CTC Glu	GCA CGT Ala * TGG ACC	* CGG GCC Arg AGC TCG	CAC Val 550 * CCC GGG Pro	TCC Arg AGC TCG Ser	GCC CGG Ala * ACC TGG	TGG ACC Trp 660 * AAG TTC Lys	CGA Ala TGG ACC	CAG GTC Gln * CAC GTG	* AGC TCG Ser 67	ATA Tyr>  O  * TCC AGG Ser>
TCT AGA Ser * AAC TTG Asn	GGG CCC Gly 630 * ACC TGG	ATT TAA Ile ACC TGG Thr	TCC AGG Ser * TGG ACC	* TAC ATG Tyr 64 AGT TCA Ser	TCC Arg 10 * GAG CTC Glu 690 *	GCA CGT Ala * TGG ACC Trp	* CGG GCC Arg AGC TCG Ser	CAC Val 550 * CCC GGG Pro	AGC TCG Ser	GCC CGG Ala * ACC TGG Thr	TGG ACC Trp 660 * AAG TTC Lys	CGA Ala TGG ACC Trp 710 *	CAG GTC Gln * CAC GTG His	* AGC TCG Ser 67 AAC TTG Asn	ATA Tyr>  O  * TCC AGG Ser>  720
TCT AGA Ser  * AAC TTG Asn  * TAC ATG	GGG CCC Gly 630 * ACC TGG Thr	ATT TAA Ile ACC TGG Thr 680 * GAG CTC	TCC AGG Ser * TGG ACC Trp	* TAC ATG TYr 64 AGT TCA Ser * TTC AAG	TCC Arg  10 * GAG CTC Glu 690 * GAG CTC	GCA CGT Ala * TGG ACC Trp	* CGG GCC Arg AGC TCG Ser  * TCC AGG	CAC Val 550 * CCC GGG Pro 70 GGT CCA	AGC TCG Ser 00 *	GCC CGG Ala * ACC TGG Thr * GGC CCG	TGG ACC Trp 660 * AAG TTC Lys GGG CCC	TGG ACC Trp 710 * GGC CCG	CAG GTC Gln * CAC GTG His	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC	ATA Tyr>  O  * TCC AGG Ser>  720  * CCT GGA
TCT AGA Ser  * AAC TTG Asn  * TAC ATG	GGG CCC Gly 630 * ACC TGG Thr	ATT TAA Ile ACC TGG Thr 680 * GAG CTC	TCC AGG Ser * TGG ACC Trp	* TAC ATG TYr 64 AGT TCA Ser * TTC AAG	TCC Arg  10 * GAG CTC Glu 690 * GAG CTC	GCA CGT Ala * TGG ACC Trp	* CGG GCC Arg AGC TCG Ser  * TCC AGG	CAC Val 550 * CCC GGG Pro 70 GGT CCA	AGC TCG Ser 00 *	GCC CGG Ala * ACC TGG Thr * GGC CCG	TGG ACC Trp 660 * AAG TTC Lys GGG CCC	TGG ACC Trp 710 * GGC CCG	CAG GTC Gln * CAC GTG His	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC	ATA Tyr> 70 * TCC AGG Ser> 720 * CCT
TCT AGA Ser  * AAC TTG Asn  * TAC ATG	GGG CCC Gly 630 * ACC TGG Thr	ATT TAA Ile ACC TGG Thr 680 * GAG CTC Glu	TCC AGG Ser * TGG ACC Trp	* TAC ATG TYr 64 AGT TCA Ser * TTC AAG	TCC Arg  10  * GAG CTC Glu  690  * GAG CTC GIu	GCA CGT Ala * TGG ACC Trp	* CGG GCC Arg AGC TCG Ser  * TCC AGG	CAC Val 550 * CCC GGG Pro 70 GGT CCA	AGC TCG Ser 00 *	GCC CGG Ala * ACC TGG Thr * GGC CCG	TGG ACC Trp 660 * AAG TTC Lys GGG CCC	TGG ACC Trp 710 * GGC CCG Gly	CAG GTC Gln * CAC GTG His	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC	ATA Tyr>  O  * TCC AGG Ser>  720  * CCT GGA
TCT AGA Ser  * AAC TTG Asn  * TAC ATG	GGG CCC Gly 630 * ACC TGG Thr	ATT TAA Ile ACC TGG Thr 680 * GAG CTC Glu	TCC AGG Ser  * TGG ACC Trp  CCC GGG Pro	* TAC ATG TYr 64 AGT TCA Ser * TTC AAG	TCC Arg  10  * GAG CTC Glu  690  * GAG CTC GIu	GCA CGT Ala * TGG ACC Trp CAG GTC Gln	* CGG GCC Arg AGC TCG Ser  * TCC AGG	CAC Val 550 * CCC GGG Pro 70 GGT CCA	AGC TCG Ser 00 * GGG CCC Gly	GCC CGG Ala * ACC TGG Thr * GGC CCG	TGG ACC Trp 660 * AAG TTC Lys GGG CCC	TGG ACC Trp 710 * GGC CCG Gly	CAG GTC Gln * CAC GTG His GCC CGG Ala	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC	ATA Tyr>  O  * TCC AGG Ser>  720  * CCT GGA
TCT AGA Ser  * AAC TTG Asn  * TAC ATG TYr	* GGG CCC Gly 630 * ACC TGG Thr AGG TCC Arg	ATT TAA Ile ACC TGG Thr 680 * GAG CTC Glu 7	TCC AGG Ser  * TGG ACC Trp  CCC GGG Pro 30 * CAG	TAC ATG Tyr  64 AGT TCA Ser  * TTC AAG Phe	TCC Arg  10 * GAG CTC Glu 690 * GAG CTC Glu CTC CTC	GCA CGT Ala  * TGG ACC Trp  CAG GTC Gln  740 * GTG	* CGG GCC Arg AGC TCG Ser  * TCC AGG Ser	CAC Val 550 * CCC GGG Pro 70 GGT CCA Gly * AAT	AGC TCG Ser  OO * GGG CCC Gly  750 *	GCC CGG Ala * ACC TGG Thr * GGC CCG Gly	TGG ACC Trp 660 * AAG TTC Lys GGG CCC Gly *	TGG ACC Trp 710 * GGC CCG Gly 7 TCT	CAG GTC Gln  * CAC GTG His  GCC CGG Ala  60  *	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC Ala	ATA Tyr>  10  * TCC AGG Ser>  720  * CCT GGA Pro>
TCT AGA Ser  * AAC TTG Asn  * TAC ATG TYT	* GGG CCC Gly 630 * ACC TGG Thr AGG TCC Arg	ATT TAA Ile  ACC TGG Thr  680 * GAG CTC Glu 77 ACT TGA	TCC AGG Ser  * TGG ACC Trp  CCC GGG Pro  CAG GTC	* TAC ATG Tyr 64 AGT TCA Ser * TTC AAG Phe CCA GGT	TCC Arg  10 * GAG CTC Glu 690 * GAG CTC Glu CCT GGA	GCA CGT Ala  * TGG ACC Trp  CAG GTC Gln  740 * GTG CAC	* CGG GCC Arg AGC TCG Ser  * TCC AGG Ser ACA	CAC Val 550 * CCC GGG Pro 70 GGT CCA Gly * AAT TTA	AGC TCG Ser  OO * GGG CCC Gly  750 * TTG	GCC CGG Ala * ACC TGG Thr * GGC CCG Gly	TGG ACC Trp 660 * AAG TTC Lys GGG CCC Gly * GTC CAG	TGG ACC Trp 710 * GGC CCG Gly 7 TCT AGA	CAG GTC Gln  * CAC GTG His  GCC CGG Ala  60  * CAA	* AGC TCG Ser 67 AAC TTG Asn * GCG CGC Ala GAA CTT	ATA Tyr>  O  * TCC AGG Ser>  720  * CCT GGA Pro>

# Fig.31C.

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770			780			79	90		8	300			810		
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CTC	TGC	ACA	GTA	ATA	TGG	ACA	TGG	AAT	CCA	CCC	GAG	GGA	GCC	AGC	TCA
GAG	ACG	TGT	CAT	TAT	ACC	TGT	ACC	TTA	GGT	GGG	CTC	CCT	CGG	TCG	AGT
Leu	Cys	Thr	Val	Ile	Trp	Thr	Trp	Asn	Pro	Pro	Glu	Glv	Ala	Ser	Ser>
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82	20		8	330			840			85	50			60	
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AAT	TGT	AGT	CTA	TGG	TAT	ተጥተጥ	AGT	САТ	ጥጥጥ	GGC	GAC	<b>א</b> א א	C A A	CAM	330
TTA	ACA	TCA	GAT	ACC	ATA	מממ	TCA	CTA	מממ	CCG	CTC	TO COLOR	CAA	CWI	MAG
Asn	Cvs	Ser	Leu	Trn	ጥኒም	Dhe	Ser.	Wic	Pho	Cly	7.50	Tira	G11	CIA	Lys>
	-1-				-7.	1 116	261	1173	FILE	GIY	ASD	гÃ2	GIII	ASP	Lys>
	870			88	3.0		\$	390			900			0.1	
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AAA	АТА	GCT	CCG	CAA	<b>እ</b> ርጥ	CCT	CCM	TC X	ת מית ת	CNA	Cms	000	ama.		
ىلىلىل	ጥልጥ	CGA	GGC	Cum	TCI	CGI	CGI	1CA	WIW	CMM	GIA	CCC	CTG	AAT	GAG
Ive	Tla	CGA	250	CII	mb	N	GCA 3	AGT	TAL	CIT	CAT	GGG	GAC	TTA	CTC
מעם	TT6	ALA	PIO	GIU	Thr	Arg	Arg	ser	тте	GIU	val	Pro	Leu	Asn	Glu>
		920			020				4.0						
*		*		•	930			94	40 *			950			960
ACC	א שעע	mcm.	CEC	~			* *	~~~				*		*	*
TCC	WYY	TGT	CIG	CAA	GIG	GGG	TCC	CAG	TGT	AGC	ACC	AAT	GAG	AGT	GAG
3	TAA	ACA	GAC	GTT	CAC	CCC	AGG	GTC	ACA	TCG	TGG	TTA	CTC	TCA	CTC
Arg	TTE	Cys	ren	GIN	val	GIA	Ser	Gln	Cys	Ser	Thr	Asn	Glu	Ser	Glu>
	*	97		•		980			990			100			
a a c	*	97	70 *	*	9	980		*	990		*	100	00 *	*	
AAG	* CCT	97 AGC	70 * ATT	* TTG	GTT	80 * GAA	AAA	* TGC	990 * ATC	TCA	* CCC	100	00 * GAA	* GGT	GAT
TTC	GGA	97 AGC TCG	70 * ATT TAA	* TTG AAC	GTT CAA	80 * GAA CTT	AAA TTT	* TGC ACG	990 * ATC TAG	TCA AGT	* CCC GGG	100 CCA GGT	OO * GAA CTT	* GGT CCA	GAT CTA
TTC	GGA	97 AGC TCG	70 * ATT TAA	* TTG AAC	GTT CAA	80 * GAA CTT	AAA TTT	* TGC ACG	990 * ATC TAG	TCA AGT	* CCC GGG	100 CCA GGT	OO * GAA CTT	* GGT CCA	GAT
TTC Lys	GGA	97 AGC TCG Ser	70 * ATT TAA Ile	* TTG AAC	GTT CAA	GAA CTT Glu	AAA TTT Lys	* TGC ACG	990 * ATC TAG Ile	TCA AGT Ser	* CCC GGG	100 CCA GGT Pro	OO * GAA CTT Glu	* GGT CCA	GAT CTA
TTC	GGA	97 AGC TCG Ser	70 * ATT TAA	* TTG AAC	GTT CAA	80 * GAA CTT	AAA TTT Lys	* TGC ACG	990 * ATC TAG Ile	TCA AGT	* CCC GGG	100 CCA GGT Pro	OO * GAA CTT	* GGT CCA	GAT CTA
TTC Lys 1010	GGA Pro	97 AGC TCG Ser	70 * ATT TAA Ile	* TTG AAC Leu	GTT CAA Val	GAA CTT Glu	AAA TTT Lys	* TGC ACG Cys	990 * ATC TAG Ile	TCA AGT Ser 040	* CCC GGG Pro	CCA GGT Pro	GAA CTT Glu	GGT CCA Gly	GAT CTA Asp>
TTC Lys 1010 * CCT	GGA Pro GAG	97 AGC TCG Ser	70 * ATT TAA Ile 1020 * GCT	* TTG AAC Leu GTG	GTT CAA Val *	GAA CTT Glu 10:	AAA TTT Lys 30 *	* TGC ACG Cys	990  * ATC TAG Ile  10 TGC	TCA AGT Ser 040 *	* CCC GGG Pro	100 CCA GGT Pro	GAA CTT Glu 1050	¢ GGT CCA Gly	GAT CTA Asp> * AGC
TTC Lys 1010 * CCT GGA	GGA Pro GAG CTC	AGC TCG Ser * TCT	ATT TAA Ile LO20 * GCT CGA	* TTG AAC Leu GTG CAC	GTT CAA Val * ACT	GAA CTT Glu 10:	AAA TTT Lys 30 * CTT GAA	* TGC ACG Cys  CAA GTT	990  * ATC TAG Ile  10 TGC ACG	TCA AGT Ser 040 * ATT TAA	* CCC GGG Pro	CCA GGT Pro	GAA CTT Glu 1050	ggt CCA Gly CTG	GAT CTA Asp> * AGC TCG
TTC Lys 1010 * CCT GGA	GGA Pro GAG CTC	AGC TCG Ser * TCT	ATT TAA Ile LO20 * GCT CGA	* TTG AAC Leu GTG CAC	GTT CAA Val * ACT	GAA CTT Glu 10:	AAA TTT Lys 30 * CTT GAA	* TGC ACG Cys  CAA GTT	990  * ATC TAG Ile  10 TGC ACG	TCA AGT Ser 040 * ATT TAA	* CCC GGG Pro	CCA GGT Pro	GAA CTT Glu 1050 *	ggt CCA Gly CTG	GAT CTA Asp> * AGC
TTC Lys 1010 * CCT GGA Pro	GGA Pro GAG CTC Glu	AGC TCG Ser * TCT	ATT TAA Ile LO20 * GCT CGA Ala	* TTG AAC Leu GTG CAC Val	GTT CAA Val * ACT	GAA CTT Glu 10: GAG CTC Glu	AAA TTT Lys 30 * CTT GAA Leu	* TGC ACG Cys  CAA GTT	990  * ATC TAG Ile  10 TGC ACG	TCA AGT Ser 040 * ATT TAA Ile	* CCC GGG Pro TGG ACC	CCA GGT Pro	GAA CTT Glu 1050 * AAC TTG Asn	ggt CCA Gly CTG GAC Leu	GAT CTA Asp> * AGC TCG
TTC Lys 1010 * CCT GGA	GGA Pro GAG CTC Glu	AGC TCG Ser * TCT AGA Ser	ATT TAA Ile LO20 * GCT CGA Ala	* TTG AAC Leu GTG CAC	GTT CAA Val * ACT	GAA CTT Glu 10: GAG CTC Glu	AAA TTT Lys 30 * CTT GAA	* TGC ACG Cys  CAA GTT	990  * ATC TAG Ile  10 TGC ACG	TCA AGT Ser 040 * ATT TAA	* CCC GGG Pro TGG ACC	CCA GGT Pro	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu	GAT CTA Asp> * AGC TCG
TTC Lys 1010 * CCT GGA Pro	GGA Pro GAG CTC Glu	AGC TCG Ser * TCT AGA Ser	ATT TAA Ile LO20 * GCT CGA Ala	* TTG AAC Leu GTG CAC Val	GTT CAA Val * ACT TGA	GAA CTT Glu 10: GAG CTC Glu	AAA TTT Lys 30 * CTT GAA Leu	* TGC ACG Cys  * CAA GTT Gln	990  * ATC TAG Ile  10 TGC ACG Cys	TCA AGT Ser 040 * ATT TAA Ile	* CCC GGG Pro TGG ACC Trp	CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu	GAT CTA Asp> * AGC TCG Ser>
TTC Lys 1010 * CCT GGA Pro 106	GGA Pro GAG CTC Glu 50 *	97 AGC TCG Ser  * TCT AGA Ser  * AAG	70  * ATT TAA Ile 1020  * GCT CGA Ala 10	* TTG AAC Leu GTG CAC Val	GTT CAA Val * ACT TGA Thr	GAA CTT Glu 103 GAG CTC Glu	AAA TTT Lys 30 * CTT GAA Leu 1080 *	* TGC ACG Cys CAA GTT Gln	990  * ATC TAG Ile  10 TGC ACG Cys	TCA AGT Ser 040 * ATT TAA Ile 109	* CCC GGG Pro TGG ACC Trp 00 *	100 CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu	GAT CTA Asp>  * AGC TCG Ser>
TTC Lys  1010  * CCT GGA Pro  106  TAC ATG	GGA Pro GAG CTC Glu * ATG TAC	97 AGC TCG Ser  * TCT AGA Ser  AAG	ATT TAA Ile LO20 * GCT CGA Ala 10 TGT ACA	TTG AAC Leu GTG CAC Val 070 * TCT AGA	GTT CAA Val * ACT TGA Thr	GAA CTT Glu 10: GAG CTC Glu *	AAA TTT Lys 30 * CTT GAA Leu 1080 *	* TGC ACG Cys CAA GTT Gln GGA CCT	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC	TCA AGT Ser 040 * ATT TAA Ile 109	* CCC GGG Pro TGG ACC Trp 0 * ACC	100 CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu L00 *	GAT CTA Asp>  * AGC TCG Ser> ACT TGA
TTC Lys  1010  * CCT GGA Pro  106  TAC ATG	GGA Pro GAG CTC Glu * ATG TAC	97 AGC TCG Ser  * TCT AGA Ser  AAG	ATT TAA Ile LO20 * GCT CGA Ala 10 TGT ACA	TTG AAC Leu GTG CAC Val 070 * TCT AGA	GTT CAA Val * ACT TGA Thr	GAA CTT Glu 10: GAG CTC Glu *	AAA TTT Lys 30 * CTT GAA Leu 1080 *	* TGC ACG Cys CAA GTT Gln GGA CCT	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC	TCA AGT Ser 040 * ATT TAA Ile 109	* CCC GGG Pro TGG ACC Trp 0 * ACC	100 CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu L00 *	GAT CTA Asp>  * AGC TCG Ser>
TTC Lys 1010 * CCT GGA Pro 106 TAC ATG Tyr	GGA Pro GAG CTC Glu * ATG TAC Met	97 AGC TCG Ser  * TCT AGA Ser  AAG	ATT TAA Ile LO20 * GCT CGA Ala 10 TGT ACA	TTG AAC Leu  GTG CAC Val  70 * TCT AGA Ser	GTT CAA Val * ACT TGA Thr TGG ACC	GAA CTT Glu 10: GAG CTC Glu *	AAA TTT Lys 30 * CTT GAA Leu 1080 * CCT GGA Pro	* TGC ACG Cys CAA GTT Gln GGA CCT Gly	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC	TCA AGT Ser 040 * ATT TAA Ile 109 AAT TTA Asn	* CCC GGG Pro TGG ACC Trp 0 * ACC TGG	100 CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn	GGT CCA Gly CTG GAC Leu L00 * GAC CTG Asp	GAT CTA Asp>  * AGC TCG Ser> ACT TGA Thr>
TTC Lys 1010 * CCT GGA Pro 106 TAC ATG Tyr	GGA Pro GAG CTC Glu * ATG TAC	97 AGC TCG Ser  * TCT AGA Ser  AAG	ATT TAA Ile LO20 * GCT CGA Ala 10 TGT ACA	TTG AAC Leu GTG CAC Val 070 * TCT AGA	GTT CAA Val * ACT TGA Thr TGG ACC	GAA CTT Glu 10: GAG CTC Glu *	AAA TTT Lys 30 * CTT GAA Leu 1080 * CCT GGA Pro	* TGC ACG Cys CAA GTT Gln GGA CCT Gly	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC	TCA AGT Ser  100 ATT TAA Ile 100 AAT TTA ASD	* CCC GGG Pro TGG ACC Trp ACC TGG Thr	100 CCA GGT Pro * CAC GTG His	GAA CTT Glu 1050 * AAC TTG Asn 1: CCC GGG	GGT CCA Gly CTG GAC Leu L00 *	GAT CTA Asp>  * AGC TCG Ser> ACT TGA Thr>
TTC Lys 1010 * CCT GGA Pro 106 TAC ATG Tyr	GGA Pro GAG CTC Glu * ATG TAC Met	AGC TCG Ser * TCT AGA Ser * AAG TTC Lys	ATT TAA Ile LO20 GCT CGA Ala 10 TGT ACA Cys	TTG AAC Leu  GTG CAC Val  70 * TCT AGA Ser	GTT CAA Val * ACT TGA Thr TGG ACC Trp	GAA CTT Glu 10: GAG CTC Glu * CTC GAG Leu	AAA TTT Lys 30 * CTT GAA Leu 1080 * CCT GGA Pro	* TGC ACG Cys CAA GTT Gln GGA CCT Gly 130 *	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC Arg	TCA AGT Ser 040 * ATT TAA Ile 109 AAT TTA Asn	* CCC GGG Pro TGG ACC Trp 0 * ACC TGG Thr	100 CCA GGT Pro * CAC GTG His * AGT TCA Ser	GAA CTT Glu 1050 * AAC TTG ASN 1: CCC GGG Pro	GGT CCA Gly CTG GAC Leu GAC CTG Asp	GAT CTA Asp>  * AGC TCG Ser> ACT TGA Thr> 50 *
TTC Lys  1010  * CCT GGA Pro  106  TAC ATG Tyr	GGA Pro GAG CTC Glu * ATG TAC Met	AGC TCG Ser  * TCT AGA Ser  AAG TTC Lys	ATT TAA Ile LO20 GCT CGA Ala 10 TGT ACA Cys	TTG AAC Leu  GTG CAC Val  70 * TCT AGA Ser 11:	GTT CAA Val  * ACT TGA Thr  TGG ACC Trp  20  * TAT	GAA CTT Glu 10: GAG CTC Glu * CTC GAG Leu	AAA TTT Lys 30 * CTT GAA Leu 1080 * CCT GGA Pro	* TGC ACG Cys CAA GTT Gln GGA CCT Gly 130 * AGA	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC Arg	TCA AGT Ser  100 ATT TAA Ile 100 AAT TTA AST CTG	* CCC GGG Pro TGG ACC Trp ACC TGG Thr	100 CCA GGT Pro * CAC GTG His * AGT TCA Ser	GAA CTT Glu 1050 * AAC TTG ASn 1: CCC GGG Pro	GGT CCA Gly CTG GAC Leu LOO * GAC CTG Asp	GAT CTA Asp>  * AGC TCG Ser> ACT TGA Thr> 50 * CAA
TTC Lys  1010  * CCT GGA Pro  106  TAC ATG Tyr  * AAC TTG	GGA Pro GAG CTC Glu 50 * ATG TAC Met 1110 * TAT	AGC TCG Ser  * TCT AGA Ser  AAG TTC Lys	ATT TAA Ile LO20 GCT CGA Ala TGT ACA Cys CTC GAG	TTG AAC Leu  GTG CAC Val  TCT AGA Ser  11: TAC ATG	GTT CAA Val * ACT TGA Thr TGG ACC Trp 20 * TAT ATA	GAA CTT Glu 10: GAG CTC Glu * CTC GAG Leu	AAA TTT Lys 30 * CTT GAA Leu 1080 * CCT GGA Pro	* TGC ACG Cys CAA GTT Gln GGA CCT Gly 130 * AGA TCT	990  * ATC TAG Ile  10 TGC ACG Cys  * AGG TCC Arg	TCA AGT Ser  100 ATT TAA Ile 100 AAT TTA Asn  * CTG GAC	* CCC GGG Pro  TGG ACC Trp  ACC TGG Thr  1140  * GAA CTT	100 CCA GGT Pro  * CAC GTG His AGT TCA Ser	GAA CTT Glu 1050 * AAC TTG ASN 1: CCC GGG Pro	GGT CCA Gly  CTG GAC Leu  GAC CTG Asp  11:  CAT GTA	GAT CTA Asp>  * AGC TCG Ser> ACT TGA Thr> 50 *

50/63

# Fig.31D.

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				TTT											
				AAA											
Cys	Glu	Asn	Ile	Phe	Arg	Glu	Gly	Gln	Tyr	Phe	Gly	Cys	Ser	Phe	Asp>
		121	.0		12	20		1	.230			124	0		
	*		*	*		*		*	*		*		*	*	
CTG	ACC	A A A	CTC	AAG	CAT	TCC	N C TT	ጥጥጥ	C 2 2	C	CAC	N C CT	CTC	C 2 2	3 m 3
				TTC											
Leu	Thr	Lys	Val	Lys	Asp	Ser	Ser	Phe	Glu	Gln	His	Ser	Val	Gln	Ile>
1250		1	.260			127	70		12	280		1	.290		
*		*	*		*		*	*		*		*	*		*
ATG	GTC	AAG	GAT	AAT	GCA	GGA	AAA	ATT	AAA	CCA	TCC	TTC	AAT	ATA	GTG
				TTA											
															Val>
	-	2,5	110p		nia	GTÅ	<b></b> y	116	Dy 3	110	Der	rne	ASII	176	va1>
130	١٨		4.5	310		4	1220			133	2.0			340	
13(	*		1.3	*		*	L320		_	13.	. ·		1.3	*	
				CGT											
				GCA											
Pro	Leu	Thr	Ser	Arg	Val	Lys	Pro	Asp	Pro	Pro	His	Ile	Lys	Asn	Leu>
1	L350			136	50		1:	370		:	1380			139	90
*	L350 *		*	136	50 *	*	1:	370 *		*	1380 *		*	139	90 *
*	*	CAC			*			*	CAA	*	*	AAT			*
* TCC	* TTC		AAT	GAT	* GAC	CTA	TAT	* GTG		* TGG	* GAG		CCA	CAG	* AAT
* TCC AGG	* TTC AAG	GTG	AAT TTA	GAT CTA	* GAC CTG	CTA GAT	TAT ATA	* GTG CAC	GTT	* TGG ACC	* GAG CTC	TTA	CCA GGT	CAG GTC	* AAT TTA
* TCC AGG	* TTC AAG	GTG	AAT TTA	GAT CTA	* GAC CTG	CTA GAT	TAT ATA	* GTG CAC	GTT	* TGG ACC	* GAG CTC	TTA	CCA GGT	CAG GTC	* AAT
* TCC AGG	* TTC AAG Phe	GTG His	AAT TTA	GAT CTA Asp	* GAC CTG Asp	CTA GAT	TAT ATA	* GTG CAC Val	GTT Gln	* TGG ACC	* GAG CTC Glu	TTA Asn	CCA GGT	CAG GTC Gln	* AAT TTA Asn>
* TCC AGG Ser	* TTC AAG Phe	GTG His 400	AAT TTA	GAT CTA Asp	* GAC CTG Asp	CTA GAT	TAT ATA	* GTG CAC	GTT Gln 20	* TGG ACC Trp	* GAG CTC Glu	TTA Asn 430	CCA GGT	CAG GTC Gln	* AAT TTA Asn>
* TCC AGG Ser	* TTC AAG Phe	GTG His 400	AAT TTA Asn	GAT CTA Asp	* GAC CTG Asp	CTA GAT Leu	TAT ATA Tyr	* GTG CAC Val	GTT Gln 20	* TGG ACC Trp	GAG CTC Glu	TTA Asn 430	CCA GGT Pro	CAG GTC Gln	* AAT TTA Asn>
* TCC AGG Ser * TTT	TTC AAG Phe 1	GTG His 400 * AGC	AAT TTA Asn	GAT CTA Asp *	* GAC CTG Asp 1410 * CTA	CTA GAT Leu TTT	TAT ATA Tyr * TAT	* GTG CAC Val 14:	GTT Gln 20 * GTA	* TGG ACC Trp  * GAA	GTC	TTA Asn 430 * AAT	CCA GGT Pro	CAG GTC Gln *	AAT TTA Asn>
* TCC AGG Ser  * TTT AAA	TTC AAG Phe  1 ATT TAA	GTG His 400 * AGC TCG	AAT TTA Asn AGA TCT	GAT CTA Asp * TGC ACG	* GAC CTG Asp 1410 * CTA GAT	CTA GAT Leu TTT AAA	TAT ATA Tyr  * TAT ATA	GAA	GTT Gln 20 * GTA CAT	* TGG ACC Trp  * GAA CTT	GAG CTC Glu  GTC CAG	TTA Asn 430 * AAT TTA	CCA GGT Pro AAC TTG	CAG GTC Gln * AGC TCG	AAT TTA Asn> L440 * CAA GTT
* TCC AGG Ser  * TTT AAA	TTC AAG Phe  1 ATT TAA	GTG His 400 * AGC TCG	AAT TTA Asn AGA TCT	GAT CTA Asp * TGC ACG	* GAC CTG Asp 1410 * CTA GAT	CTA GAT Leu TTT AAA	TAT ATA Tyr  * TAT ATA	GAA	GTT Gln 20 * GTA CAT	* TGG ACC Trp  * GAA CTT	GAG CTC Glu  GTC CAG	TTA Asn 430 * AAT TTA	CCA GGT Pro AAC TTG	CAG GTC Gln * AGC TCG	AAT TTA Asn>
* TCC AGG Ser  * TTT AAA	TTC AAG Phe  1 ATT TAA	GTG His 400 * AGC TCG	AAT TTA Asn AGA TCT	GAT CTA Asp * TGC ACG	* GAC CTG Asp 1410 * CTA GAT	CTA GAT Leu TTT AAA	TAT ATA Tyr  * TAT ATA	GAA	GTT Gln 20 * GTA CAT	* TGG ACC Trp  * GAA CTT	GAG CTC Glu  GTC CAG	TTA Asn 430 * AAT TTA	CCA GGT Pro AAC TTG	CAG GTC Gln * AGC TCG	AAT TTA Asn> L440 * CAA GTT
* TCC AGG Ser  * TTT AAA	TTC AAG Phe  1 ATT TAA	GTG His 400 * AGC TCG	AAT TTA Asn AGA TCT Arg	GAT CTA Asp * TGC ACG	GAC CTG Asp 1410 * CTA GAT Leu	CTA GAT Leu TTT AAA	TAT ATA Tyr  * TAT ATA	* GTG CAC Val  14: GAA CTT Glu	GTT Gln 20 * GTA CAT	TGG ACC Trp * GAA CTT	GAG CTC Glu  GTC CAG	TTA Asn 430 * AAT TTA	CCA GGT Pro AAC TTG Asn	CAG GTC Gln * AGC TCG	AAT TTA Asn> L440 * CAA GTT
* TCC AGG Ser  * TTT AAA	TTC AAG Phe  1 ATT TAA	GTG His 400 * AGC TCG Ser	AAT TTA Asn AGA TCT Arg	GAT CTA Asp * TGC ACG	GAC CTG Asp 1410 * CTA GAT Leu	CTA GAT Leu TTT AAA Phe	TAT ATA Tyr  * TAT ATA	* GTG CAC Val  14: GAA CTT Glu	GTT Gln 20 * GTA CAT Val	TGG ACC Trp * GAA CTT	GAG CTC Glu  GTC CAG	TTA Asn 430 * AAT TTA Asn	CCA GGT Pro AAC TTG Asn	CAG GTC Gln * AGC TCG	AAT TTA Asn> L440 * CAA GTT
* TCC AGG Ser  * TTT AAA Phe	* TTC AAG Phe  1 ATT TAA Ile	GTG His 400 * AGC TCG Ser	AAT TTA Asn AGA TCT Arg	GAT CTA Asp * TGC ACG Cys	GAC CTG Asp 1410 * CTA GAT Leu	CTA GAT Leu TTT AAA Phe	TAT ATA Tyr  * TAT ATA Tyr	GAA CTT Glu	GTT Gln 20 * GTA CAT Val 1470	* TGG ACC Trp  * GAA CTT Glu	GAG CTC Glu  1. GTC CAG Val	TTA Asn 430 * AAT TTA Asn	CCA GGT Pro AAC TTG Asn 80	CAG GTC Gln * AGC TCG Ser	AAT TTA Asn> 1440  CAA GTT Gln>
TCC AGG Ser  * TTT AAA Phe	* TTC AAG Phe  1 ATT TAA Ile  * GAG	GTG His 400 * AGC TCG Ser 14	AAT TTA Asn AGA TCT Arg 50 *	GAT CTA Asp * TGC ACG Cys	GAC CTG Asp 1410 * CTA GAT Leu	CTA GAT Leu TTT AAA Phe 460 * TTC	TAT ATA Tyr  * TAT ATA Tyr  TAC	GTG CAC Val  14: GAA CTT Glu  * GTC	GTT Gln 20 * GTA CAT Val 1470 *	TGG ACC Trp  * GAA CTT Glu	* GAG CTC Glu  1 GTC CAG Val  * GCT	TTA Asn 430 * AAT TTA Asn 14	CCA GGT Pro AAC TTG Asn 80	CAG GTC Gln * AGC TCG Ser	AAT TTA Asn> 1440 * CAA GTT Gln>
TCC AGG Ser  * TTT AAA Phe  ACT TGA	* TTC AAG Phe  1 ATT TAA Ile  * GAG CTC	GTG His 400 * AGC TCG Ser 14 ACA TGT	AAT TTA Asn AGA TCT Arg 50 *	GAT CTA Asp * TGC ACG Cys	GAC CTG Asp 410 * CTA GAT Leu GTT CAA	CTA GAT Leu TTT AAA Phe 460 * TTC AAG	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG	GTG CAC Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT	TGG ACC Trp  * GAA CTT Glu  GAG CTC	GAG CTC Glu  1 GTC CAG Val  * GCT CGA	TTA Asn 430 * AAT TTA Asn 14 AAA TTT	CCA GGT Pro AAC TTG Asn *	CAG GTC Gln * AGC TCG Ser *	AAT TTA Asn> L440 * CAA GTT Gln> AAT TTA
TCC AGG Ser  * TTT AAA Phe  ACT TGA	* TTC AAG Phe  1 ATT TAA Ile  * GAG CTC	GTG His 400 * AGC TCG Ser 14 ACA TGT	AAT TTA Asn AGA TCT Arg 50 *	GAT CTA Asp * TGC ACG Cys	GAC CTG Asp 410 * CTA GAT Leu GTT CAA	CTA GAT Leu TTT AAA Phe 460 * TTC AAG	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG	GTG CAC Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT	TGG ACC Trp  * GAA CTT Glu  GAG CTC	GAG CTC Glu  1 GTC CAG Val  * GCT CGA	TTA Asn 430 * AAT TTA Asn 14 AAA TTT	CCA GGT Pro AAC TTG Asn *	CAG GTC Gln * AGC TCG Ser *	AAT TTA Asn> 1440 * CAA GTT Gln>
TCC AGG Ser  * TTT AAA Phe  ACT TGA Thr	* TTC AAG Phe  1 ATT TAA Ile  * GAG CTC	GTG His  400  * AGC TCG Ser  14  ACA TGT Thr	AAT TTA Asn AGA TCT Arg 50 * CAT GTA His	GAT CTA Asp * TGC ACG Cys * AAT TTA Asn	GAC CTG Asp 410 * CTA GAT Leu GTT CAA	CTA GAT Leu TTT AAA Phe 460 * TTC AAG Phe	TAT ATA TYT  * TAT ATA TYT  TAC ATG	GTG CAC Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT Gln	* TGG ACC Trp  * GAA CTT Glu GAG CTC	GAG CTC Glu  1 GTC CAG Val  * GCT CGA	TTA Asn 430 * AAT TTA Asn 14 AAA TTT	CCA GGT Pro AAC TTG ASN * TGT ACA Cys	CAG GTC Gln * AGC TCG Ser * GAG CTC	AAT TTA Asn> L440 * CAA GTT Gln> AAT TTA
TCC AGG Ser  * TTT AAA Phe  ACT TGA	* TTC AAG Phe  1 ATT TAA Ile  * GAG CTC	GTG His  400  * AGC TCG Ser  14  ACA TGT Thr	AAT TTA Asn AGA TCT Arg 50 *	GAT CTA Asp * TGC ACG Cys * AAT TTA Asn	GAC CTG Asp 410 * CTA GAT Leu GTT CAA	CTA GAT Leu TTT AAA Phe 460 * TTC AAG Phe	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG	GTG CAC Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT Gln	TGG ACC Trp  * GAA CTT Glu  GAG CTC	GAG CTC Glu  1 GTC CAG Val  * GCT CGA	TTA Asn 430 * AAT TTA Asn 14 AAA TTT	CCA GGT Pro AAC TTG Asn *	CAG GTC Gln * AGC TCG Ser * GAG CTC	AAT TTA Asn> L440 * CAA GTT Gln> AAT TTA
TCC AGG Ser  * TTT AAA Phe  ACT TGA Thr	TTC AAG Phe  ATT TAA Ile  * GAG CTC Glu	GTG His  400 * AGC TCG Ser 14 ACA TGT Thr	AAT TTA Asn AGA TCT Arg 50 * CAT GTA His	GAT CTA Asp * TGC ACG Cys * AAT TTA Asn	GAC CTG Asp 1410 * CTA GAT Leu 1. GTT CAA Val	CTA GAT Leu TTT AAA Phe 460 * TTC AAG Phe	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG Tyr  10 *	GTG CAC Val  14: GAA CTT Glu  * GTC CAG Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT Gln	TGG ACC Trp  * GAA CTT Glu  GAG CTC Glu  520 *	GAG CTC Glu  1 GTC CAG Val  * GCT CGA Ala	TTA Asn 430 * AAT TTA Asn 14 AAA TTT Lys	CCA GGT Pro AAC TTG ASN * TGT ACA Cys	CAG GTC Gln * AGC TCG Ser * GAG CTC Glu	AAT TTA Asn> 1440 * CAA GTT Gln> AAT TTA Asn>
* TCC AGG Ser  * TTT AAA Phe  ACT TGA Thr  1490 * CCA	TTC AAG Phe  1 ATT TAA Ile  * GAG CTC Glu	GTG His  400 * AGC TCG Ser 14 ACA TGT Thr	AAT TTA Asn AGA TCT Arg 50 * CAT GTA His	GAT CTA Asp * TGC ACG Cys * AAT TTA Asn	GAC CTG Asp 410 * CTA GAT Leu 1. GTT CAA Val	CTA GAT Leu TTT AAA Phe 460 * TTC AAG Phe 15	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG Tyr  10 * GAG	GTG CAC Val  14: GAA CTT Glu  * GTC CAG Val	GTT Gln 20 * GTA CAT Val 1470 * CAA GTT Gln	TGG ACC Trp  * GAA CTT Glu  GAG CTC Glu  520 *	GAG CTC Glu  1 GTC CAG Val  * GCT CGA Ala	TTA Asn 430 * AAT TTA Asn 14 AAA TTT Lys *	CCA GGT Pro AAC TTG Asn * TGT ACA Cys	CAG GTC Gln  * AGC TCG Ser  GAG CTC Glu	AAT TTA Asn> L440 * CAA GTT Gln> AAT TTA Asn>
* TCC AGG Ser  * TTT AAA Phe  ACT TGA Thr  1490 * CCA GGT	TTC AAG Phe  1 ATT TAA Ile  * GAG CTC Glu	GTG His  400  AGC TCG Ser  14  ACA TGT Thr	AAT TTA Asn AGA TCT Arg 50 * CAT GTA His	GAT CTA Asp * TGC ACG Cys * AAT TTA Asn	GAC CTG Asp 1410  CTA GAT Leu  CTA CAA Val  AAT	CTA GAT Leu TTT AAA Phe 460 * TTC AAG Phe 15	TAT ATA Tyr  * TAT ATA Tyr  TAC ATG Tyr  10  * GAG	GTG CAC Val 14: GAA CTT Glu * GTC CAG Val AAT TTA	GTT Gln  20 * GTA CAT Val  1470 * CAA GTT Gln  1 ACA	* TGG ACC Trp  * GAA CTT Glu GAG CTC Glu 520 * TCT AGA	GAG CTC Glu  1 GTC CAG Val  * GCT CGA Ala	TTA Asn 430 * AAT TTA Asn 14 AAA TTT Lys * TTC	CCA GGT Pro AAC TTG Asn * TGT ACA Cys	CAG GTC Gln  * AGC TCG Ser  GAG CTC Glu	AAT TTA Asn> 1440 * CAA GTT Gln> AAT TTA Asn>

# Fig.31E.

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	033	100				*		*		*	*		*		*	*
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	Glu	Met	Ser	Ile	Gly	Lys	Lys	Arg	Asn	Ser	Thr	Thr	Gly	Asp	Lys	Thr>
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17	30		1	L740			175	50		17	760		1	L <b>77</b> 0		
17	'30 *		*	L740 *		*	175	50 *	*	17	760 *		*	L770 *		*
17	*	TTC	*	*	ccc	* CCA		*			*	СТС	*	*	ጥርር	*
17	* GTC	TTC AAG	* CTC	* TTC	CCC	* CCA GGT	AAA	* CCC	AAG	GAC	* ACC	CTC	* ATG	* ATC	TCC	* CGG
17	* GTC CAG	AAG	* CTC GAG	* TTC AAG	GGG	GGT	AAA TTT	* CCC GGG	AAG TTC	GAC CTG	* ACC TGG	GAG	* ATG TAC	* ATC TAG	AGG	GCC
17	* GTC CAG	AAG	* CTC GAG	* TTC AAG	GGG	GGT	AAA TTT	* CCC GGG	AAG TTC	GAC CTG	* ACC TGG	GAG	* ATG TAC	* ATC TAG	AGG	* CGG GCC Arg>
17	* GTC CAG	AAG Phe	* CTC GAG	* TTC AAG Phe	GGG	GGT	AAA TTT Lys	* CCC GGG Pro	AAG TTC	GAC CTG	* ACC TGG Thr	GAG Leu	* ATG TAC	* ATC TAG Ile	AGG Ser	GCC
17	* GTC CAG Val	AAG Phe	* CTC GAG	* TTC AAG Phe	GGG Pro	GGT	AAA TTT Lys	* CCC GGG	AAG TTC	GAC CTG	* ACC TGG	GAG Leu	* ATG TAC Met	* ATC TAG Ile	AGG Ser 320	GCC
17	* GTC CAG Val	AAG Phe 30	* CTC GAG Leu	* TTC AAG Phe	GGG Pro 790 *	GGT Pro	AAA TTT Lys	* CCC GGG Pro	AAG TTC Lys	GAC CTG Asp	* ACC TGG Thr	GAG Leu LO	* ATG TAC Met	ATC TAG Ile	AGG Ser 320	GCC Arg>
17	* GTC CAG Val 178	AAG Phe 30 *	* CTC GAG Leu * GAG	* TTC AAG Phe 17	GGG Pro 790 * ACA	GGT Pro	AAA TTT Lys *	* CCC GGG Pro L800 *	AAG TTC Lys GTG	GAC CTG Asp *	* ACC TGG Thr 181	GAG Leu LO *	* ATG TAC Met	ATC TAG Ile	AGG Ser 320 *	GCC Arg>
17	* GTC CAG Val 178 ACC TGG	AAG Phe  80 * CCT GGA	* CTC GAG Leu  * GAG CTC	TTC AAG Phe 17 GTC CAG	GGG Pro 790 * ACA TGT	GGT Pro TGC ACG	AAA TTT Lys * GTG CAC	CCC GGG Pro L800 * GTG CAC	AAG TTC Lys GTG CAC	GAC CTG Asp * GAC CTG	* ACC TGG Thr 181 GTG CAC	GAG Leu LO * AGC TCG	* ATG TAC Met  CAC GTG	ATC TAG Ile 18	AGG Ser 320 * GAC CTG	GCC Arg> CCT GGA
17	* GTC CAG Val 178 ACC TGG	AAG Phe  80 * CCT GGA	* CTC GAG Leu  * GAG CTC	TTC AAG Phe 17 GTC CAG	GGG Pro 790 * ACA TGT	GGT Pro TGC ACG	AAA TTT Lys * GTG CAC	CCC GGG Pro L800 * GTG CAC	AAG TTC Lys GTG CAC	GAC CTG Asp * GAC CTG	* ACC TGG Thr 181 GTG CAC	GAG Leu LO * AGC TCG	* ATG TAC Met  CAC GTG	ATC TAG Ile 18	AGG Ser 320 * GAC CTG	GCC Arg>
17	* GTC CAG Val 178 ACC TGG	AAG Phe 30 * CCT GGA Pro	* CTC GAG Leu  * GAG CTC	TTC AAG Phe 17 GTC CAG	GGG Pro 790 * ACA TGT Thr	TGC ACG Cys	AAA TTT Lys * GTG CAC	CCC GGG Pro L800 * GTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * GAC CTG	ACC TGG Thr 181 GTG CAC Val	GAG Leu LO * AGC TCG Ser	* ATG TAC Met  CAC GTG	ATC TAG Ile 18	AGG Ser 320 * GAC CTG Asp	GCC Arg> CCT GGA Pro>
17	* GTC CAG Val 178 ACC TGG	AAG Phe  80 * CCT GGA	* CTC GAG Leu  * GAG CTC	TTC AAG Phe 17 GTC CAG	GGG Pro 790 * ACA TGT	TGC ACG Cys	AAA TTT Lys * GTG CAC	CCC GGG Pro L800 * GTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * GAC CTG	ACC TGG Thr 181 GTG CAC Val	GAG Leu LO * AGC TCG Ser	* ATG TAC Met  CAC GTG	ATC TAG Ile 18 GAA CTT Glu	AGG Ser 320 * GAC CTG	GCC Arg> CCT GGA Pro>
17	* GTC CAG Val 178 ACC TGG Thr	AAG Phe 30 * CCT GGA Pro .830 *	* CTC GAG Leu  * GAG CTC Glu	* TTC AAG Phe 17 GTC CAG Val	GGG Pro 790 * ACA TGT Thr	TGC ACG Cys	AAA TTT Lys * GTG CAC Val	CCC GGG Pro L800 * GTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * GAC CTG Asp	* ACC TGG Thr 181 GTG CAC Val	GAG Leu LO * AGC TCG Ser L860	* ATG TAC Met  CAC GTG His	ATC TAG Ile 18 GAA CTT Glu	AGG Ser 320 * GAC CTG Asp	GCC Arg> CCT GGA Pro>
17	* GTC CAG Val 178 ACC TGG Thr	AAG Phe 30 * CCT GGA Pro .830 *	* CTC GAG Leu  * GAG CTC Glu	* TTC AAG Phe 17 GTC CAG Val	GGG Pro 790 * ACA TGT Thr 184	GGT Pro TGC ACG Cys	AAA TTT Lys * GTG CAC Val	CCC GGG Pro L800 * GTG CAC Val	AAG TTC Lys GTG CAC Val	GAC CTG Asp * GAC CTG Asp	* ACC TGG Thr 181 GTG CAC Val  * GTG	GAG Leu LO * AGC TCG Ser L860 *	* ATG TAC Met  CAC GTG His	ATC TAG Ile 18 GAA CTT Glu *	AGG Ser 320 * GAC CTG Asp	GCC Arg> CCT GGA Pro>
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG	* CTC GAG Leu  * GAG CTC Glu  AAG TTC	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG	GGG Pro 790 * ACA TGT Thr 184 AAC	TGC ACG Cys TGG ACG ACG	AAA TTT Lys  * GTG CAC Val  * TAC ATG	CCC GGG Pro 1800 * GTG CAC Val 18	AAG TTC Lys GTG CAC Val 850 * GAC CTG	GAC CTG Asp * GAC CTG Asp	* ACC TGG Thr 181 GTG CAC Val  * GTG CAC	GAG Leu LO * AGC TCG Ser L860 * GAG	* ATG TAC Met  * CAC GTG His	* ATC TAG Ile  18 GAA CTT Glu  * CAT	AGG Ser 320 * GAC CTG Asp 187	GCC Arg>  CCT GGA Pro>  CCT GGC
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG	* CTC GAG Leu  * GAG CTC Glu  AAG TTC	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG	GGG Pro 790 * ACA TGT Thr 184 AAC	TGC ACG Cys TGG ACG ACG	AAA TTT Lys  * GTG CAC Val  * TAC ATG	CCC GGG Pro 1800 * GTG CAC Val 18	AAG TTC Lys GTG CAC Val 850 * GAC CTG	GAC CTG Asp * GAC CTG Asp	* ACC TGG Thr 181 GTG CAC Val  * GTG CAC	GAG Leu LO * AGC TCG Ser L860 * GAG	* ATG TAC Met  * CAC GTG His	* ATC TAG Ile  18 GAA CTT Glu  * CAT	AGG Ser 320 * GAC CTG Asp 187	GCC Arg> CCT GGA Pro>
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG Val	* CTC GAG Leu  * GAG CTC Glu  AAG TTC Lys	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG	GGG Pro 790 * ACA TGT Thr 184 AAC TTG Asn	TGC ACG Cys 10 * TGG ACC	AAA TTT Lys  * GTG CAC Val  * TAC ATG	CCC GGG Pro 1800 * GTG CAC Val 18	AAG TTC Lys GTG CAC Val * GAC CTG Asp	GAC CTG Asp * GAC CTG Asp GGC CCG Gly	* ACC TGG Thr 181 GTG CAC Val  * GTG CAC	GAG Leu 10 * AGC TCG Ser 1860 * GAG CTC Glu	* ATG TAC Met  * CAC GTG His GTG CAC Val	* ATC TAG Ile  18 GAA CTT Glu  * CAT	AGG Ser 320 * GAC CTG Asp 187 AAT TTA	CCT GGA Pro> GCC CGG Ala>
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG Val	* CTC GAG Leu  * GAG CTC Glu  AAG TTC	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG	GGG Pro 790 * ACA TGT Thr 184 AAC TTG Asn	TGC ACG Cys TGG ACG ACG	AAA TTT Lys  * GTG CAC Val  * TAC ATG	CCC GGG Pro 1800 * GTG CAC Val 18	AAG TTC Lys GTG CAC Val 850 * GAC CTG	GAC CTG Asp * GAC CTG Asp GGC CCG Gly	* ACC TGG Thr 181 GTG CAC Val  * GTG CAC	GAG Leu 10 * AGC TCG Ser 1860 * GAG CTC Glu	* ATG TAC Met  * CAC GTG His	* ATC TAG Ile  18 GAA CTT Glu  * CAT	AGG Ser 320 * GAC CTG Asp 187 AAT TTA	GCC Arg>  CCT GGA Pro>  GCC * GCC
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC Glu	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG Val	* CTC GAG Leu  * GAG CTC Glu  AAG TTC Lys 880 *	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG Phe	GGG Pro 790 * ACA TGT Thr 184 AAC TTG Asn	TGC ACG Cys TGG ACC Trp	AAA TTT Lys * GTG CAC Val * TAC ATG	CCC GGG Pro 1800 * GTG CAC Val GTG CAC Val	AAG TTC Lys GTG CAC Val * GAC CTG Asp	GAC CTG Asp * GAC CTG Asp GGC CCG Gly	* ACC TGG Thr  181 GTG CAC Val  * GTG CAC Val  *	GAG Leu LO * AGC TCG Ser L860 * GAG CTC Glu	* ATG TAC Met  CAC GTG His  GTG CAC Val	ATC TAG Ile  18 GAA CTT Glu  * CAT GTA His	AGG Ser 320 * GAC CTG Asp 187 TTA AST	CCT GGA Pro> GCC CGG Ala>
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC Glu * AAG	AAG Phe 30 * CCT GGA Pro .830 * GTC CAG Val 18	* CTC GAG Leu  * GAG CTC Glu  AAG TTC Lys 880 * AAG	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG Phe	GGG Pro 790 * ACA TGT Thr 184 AAC TTG ASN	TGC ACG Cys TGG ACC TTP	AAA TTT Lys  * GTG CAC Val  * TAC ATG TYT	CCC GGG Pro 1800 * GTG CAC Val GTG CAC Val	AAG TTC Lys GTG CAC Val S50 * GAC CTG Asp	GAC CTG Asp * GAC CTG Asp GGC CCG Gly	* ACC TGG Thr  181 GTG CAC Val  * GTG CAC Val  AGC	GAG Leu LO * AGC TCG Ser L860 * GAG CTC Glu	* ATG TAC Met  * CAC GTG His GTG CAC Val	ATC TAG Ile  18 GAA CTT Glu  * CAT GTA His	AGG Ser 320 * GAC CTG Asp 187 TTA AST	CCT GGA Pro> GCC CGG Ala>
17	* GTC CAG Val 178 ACC TGG Thr  * GAG CTC Glu  * AAG TTC	AAG Phe  CCT GGA Pro  .830 * GTC CAG Val  18 ACA TGT	* CTC GAG Leu  * GAG CTC Glu  AAG TTC Lys  880 * AAG TTC	* TTC AAG Phe  17 GTC CAG Val  * TTC AAG Phe  CCG GGC	GGG Pro  790  * ACA TGT Thr  184  AAC TTG ASn  * CGG GCC	TGC ACG Cys TGG ACC Trp L890 CAG CTC	AAA TTT Lys  * GTG CAC Val  * TAC ATG Tyr  GAG CTC	CCC GGG Pro 1800 * GTG CAC Val 18 GTG CAC Val	AAG TTC Lys GTG CAC Val S50 * GAC CTG Asp 190 TAC ATG	GAC CTG Asp * GAC CTG Asp GGC CCG Gly	* ACC TGG Thr  181 GTG CAC Val  * GTG CAC Val  AGC TCG	GAG Leu LO * AGC TCG Ser L860 CTC GLu ACG TGC	* ATG TAC Met  * CAC GTG His GTG CAC Val  10  TAC	ATC TAG Ile  18 GAA CTT Glu  * CAT GTA His	AGG Ser  320  GAC CTG Asp  187 TTA Asn  * GTG	CCT GGA Pro>  GCC CGG Ala>

# Fig.31F.

		19:	30		19	940		:	1950			196	50		
	*		*	*		*		*	*		*		*	*	
		CTC													
TCG	CAG	GAG	TGG	CAG	GAC	GTG	GTC	CTG	ACC	GAC	TTA	CCG	TTC	CTC	ATG
261	vaı	rea	THE	vaı	Leu	His	GIn	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr>
1970		:	1980			199	90		20	000		2	2010		
*		*	*		*		*	*	ω.	*		*	*		*
AAG	TGC	AAG	GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC
TTC	ACG	TTC	CAG	AGG	TTG	TTT	CGG	GAG	GGT	CGG	GGG	TAG	CTC	TTT	TGG
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr>
202	2.0		20	030		-	2040			205	: n		2.0		
	*	*	2 (	*		*	*		*	20:	*	*	2(	)60 *	
ATC	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG
		TTT													
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu>
•	2070			200										•	
*	*		*	208	*	*	20	90		*	2100		*	211	ro
CCC	CCA	TCC	CGG	GAG	GAG	ATG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC
		AGG													
															Cys>
				_				•							
•	21	L20			2130			214		-4-	21	.50		. 2	2160
* CTG		*	GGC	*	*	CCC	*		*	*		*	mcc.	*	*
	GTC	* AAA		* TTC	* TAT		AGC	GAC	* ATC	GCC	GTG	* GAG		* GAG	* AGC
GAC	GTC CAG	* AAA TTT	CCG	* TTC AAG	* TAT ATA	GGG	AGC TCG	GAC CTG	* ATC TAG	GCC CGG	GTG CAC	* GAG CTC	ACC	* GAG CTC	* AGC TCG
GAC	GTC CAG	* AAA TTT	CCG	* TTC AAG	* TAT ATA	GGG	AGC TCG	GAC CTG	* ATC TAG	GCC CGG	GTG CAC	* GAG CTC	ACC	* GAG CTC	* AGC
GAC	GTC CAG	* AAA TTT	CCG Gly	* TTC AAG Phe	* TAT ATA Tyr	GGG	AGC TCG	GAC CTG Asp	* ATC TAG	GCC CGG	GTG CAC	* GAG CTC	ACC Trp	* GAG CTC	* AGC TCG
GAC Leu	GTC CAG Val	* AAA TTT Lys	CCG Gly 70 *	* TTC AAG Phe	TAT ATA Tyr	GGG Pro	AGC TCG Ser	GAC CTG Asp	* ATC TAG Ile 2190 *	GCC CGG Ala	GTG CAC Val	* GAG CTC Glu 220	ACC Trp	* GAG CTC Glu *	* AGC TCG Ser>
GAC Leu AAT	GTC CAG Val	* AAA TTT Lys 21	CCG Gly 70 * CCG	* TTC AAG Phe  * GAG	TAT ATA TYT  21	GGG Pro L80 *	AGC TCG Ser	GAC CTG Asp *	* ATC TAG Ile 2190 * ACC	GCC CGG Ala	GTG CAC Val	GAG CTC Glu 220	ACC Trp 00 * GTG	* GAG CTC Glu  * CTG	* AGC TCG Ser>
GAC Leu AAT TTA	GTC CAG Val * GGG CCC	* AAA TTT Lys 21: CAG GTC	CCG Gly 70 * CCG GGC	* TTC AAG Phe  * GAG CTC	TAT ATA Tyr  21  AAC TTG	GGG Pro L80 * AAC TTG	AGC TCG Ser TAC ATG	GAC CTG Asp * AAG TTC	* ATC TAG Ile 2190 * ACC TGG	GCC CGG Ala ACG TGC	GTG CAC Val * CCT GGA	GAG CTC Glu 220 CCC GGG	ACC Trp 00 * GTG CAC	* GAG CTC Glu * CTG GAC	* AGC TCG Ser> GAC CTG
GAC Leu AAT TTA	GTC CAG Val * GGG CCC	* AAA TTT Lys 21: CAG GTC	CCG Gly 70 * CCG GGC	* TTC AAG Phe  * GAG CTC	TAT ATA Tyr  21  AAC TTG	GGG Pro L80 * AAC TTG	AGC TCG Ser TAC ATG	GAC CTG Asp * AAG TTC	* ATC TAG Ile 2190 * ACC TGG	GCC CGG Ala ACG TGC	GTG CAC Val * CCT GGA	GAG CTC Glu 220 CCC GGG	ACC Trp 00 * GTG CAC	* GAG CTC Glu * CTG GAC	* AGC TCG Ser>
GAC Leu AAT TTA	GTC CAG Val * GGG CCC	* AAA TTT Lys 21: CAG GTC GIn	CCG Gly 70 * CCG GGC	* TTC AAG Phe  * GAG CTC	TAT ATA Tyr  21  AAC TTG	GGG Pro L80 * AAC TTG	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC	ATC TAG Ile 2190 * ACC TGG	GCC CGG Ala ACG TGC	GTG CAC Val * CCT GGA	* GAG CTC Glu 220 CCC GGG Pro	ACC Trp 00 * GTG CAC	* GAG CTC Glu * CTG GAC	* AGC TCG Ser> GAC CTG
GAC Leu AAT TTA Asn 2210	GTC CAG Val * GGG CCC Gly	* AAA TTT Lys 21: CAG GTC Gln *	CCG Gly 70 * CCG GGC Pro	* TTC AAG Phe  * GAG CTC Glu	* TAT ATA Tyr 21 AAC TTG Asn	GGG Pro L80 * AAC TTG Asn	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys	ATC TAG Ile 2190 * ACC TGG Thr	GCC CGG Ala ACG TGC Thr	GTG CAC Val * CCT GGA Pro	* GAG CTC Glu 220 CCC GGG Pro	ACC Trp 00 * GTG CAC Val 2250	* GAG CTC Glu  * CTG GAC Leu	* AGC TCG Ser> GAC CTG Asp>
GAC Leu AAT TTA Asn 2210	GTC CAG Val * GGG CCC Gly	* AAA TTT Lys 21 CAG GTC GIn * GGC	CCG Gly 70 * CCG GGC Pro 2220 *	* TTC AAG Phe  * GAG CTC Glu	TAT ATA Tyr  21  AAC TTG Asn  * TTC	GGG Pro 180 * AAC TTG Asn 223	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys	ATC TAG Ile 2190 ACC TGG Thr 22	GCC CGG Ala ACG TGC Thr	GTG CAC Val * CCT GGA Pro	GAG CTC Glu 220 CCC GGG Pro * GTG	ACC Trp 00 * GTG CAC Val 2250 *	* GAG CTC Glu  * CTG GAC Leu	* AGC TCG Ser> GAC CTG Asp> * AGC
AAT TTA Asn 2210 * TCC AGG	GTC CAG Val * GGG CCC Gly	* AAA TTT Lys 21 CAG GTC GIn * GGC CCG	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG	* TTC AAG Phe  * GAG CTC Glu  TTC AAG	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG	GGG Pro 180 * AAC TTG Asn 223 CTC GAG	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys AGC TCG	ATC TAG Ile 2190 * ACC TGG Thr 2:	GCC CGG Ala ACG TGC Thr 240 * CTC GAG	GTG CAC Val * CCT GGA Pro	GAG CTC Glu 220 CCC GGG Pro * GTG CAC	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG	* GAG CTC Glu  * CTG GAC Leu  AAG TTC	* AGC TCG Ser> GAC CTG Asp> * AGC TCG
AAT TTA Asn 2210 * TCC AGG	GTC CAG Val * GGG CCC Gly	* AAA TTT Lys 21 CAG GTC GIn * GGC CCG	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG	* TTC AAG Phe  * GAG CTC Glu  TTC AAG	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG	GGG Pro 180 * AAC TTG Asn 223 CTC GAG	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys AGC TCG	ATC TAG Ile 2190 * ACC TGG Thr 2:	GCC CGG Ala ACG TGC Thr 240 * CTC GAG	GTG CAC Val * CCT GGA Pro	GAG CTC Glu 220 CCC GGG Pro * GTG CAC	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG	* GAG CTC Glu  * CTG GAC Leu  AAG TTC	* AGC TCG Ser> GAC CTG Asp> * AGC
AAT TTA Asn 2210 * TCC AGG	GTC CAG Val * GGG CCC Gly GAC CTG Asp	* AAA TTT Lys 21 CAG GTC GIn * GGC CCG	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG Ser	* TTC AAG Phe  * GAG CTC Glu  TTC AAG	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG	GGG Pro 180 * AAC TTG Asn 223 CTC GAG Leu	AGC TCG Ser TAC ATG Tyr	GAC CTG Asp * AAG TTC Lys AGC TCG	ATC TAG Ile 2190 * ACC TGG Thr 2:	GCC CGG Ala ACG TGC Thr 240 * CTC GAG	GTG CAC Val * CCT GGA Pro	GAG CTC Glu 220 CCC GGG Pro * GTG CAC	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG Asp	* GAG CTC Glu  * CTG GAC Leu  AAG TTC	AGC TCG Ser> GAC CTG Asp>  * AGC TCG
AAT TTA Asn 2210 * TCC AGG Ser	GTC CAG Val * GGG CCC Gly GAC CTG Asp	AAA TTT Lys 21° CAG GTC GIn * GGC CCG Gly	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG Ser	* TTC AAG Phe  * GAG CTC Glu  TTC AAG Phe	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG Phe	GGG Pro 180 * AAC TTG Asn 223 CTC GAG Leu	AGC TCG Ser TAC ATG Tyr 30 * TAT ATA Tyr	GAC CTG Asp * AAG TTC Lys AGC TCG Ser	ATC TAG Ile 2190 * ACC TGG Thr 2: AAG TTC Lys	GCC CGG Ala ACG TGC Thr 240 * CTC GAG Leu	GTG CAC Val  * CCT GGA Pro  ACC TGG Thr	GAG CTC Glu  220 CCC GGG Pro  * GTG CAC Val	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG Asp	CTG GAC Leu  AAG TTC Lys	* AGC TCG Ser> GAC CTG Asp> * AGC TCG Ser>
GAC Leu AAT TTA Asn 2210 * TCC AGG Ser 226	GTC CAG Val * GGG CCC Gly GAC CTG Asp	AAA TTT Lys 21 CAG GTC GIn * GGC CCG Gly * CAG	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG Ser 22	* TTC AAG Phe  * GAG CTC Glu  TTC AAG Phe 270 * GGG	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG Phe	GGG Pro 180 * AAC TTG Asn 223 CTC GAG Leu	AGC TCG Ser  TAC ATG Tyr  TAT ATA Tyr  2280  *	GAC CTG Asp * AAG TTC Lys AGC TCG Ser	ATC TAG Ile 2190 * ACC TGG Thr 2: AAG TTC Lys	GCC CGG Ala ACG TGC Thr 240 * CTC GAG Leu 229	GTG CAC Val  * CCT GGA Pro  ACC TGG Thr	GAG CTC Glu 220 CCC GGG Pro * GTG CAC Val	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG Asp	CTG GAC Leu  AAG TTC Lys  GAG  GAG	* AGC TCG Ser> GAC CTG Asp>  * AGC TCG Ser>
GAC Leu AAT TTA Asn 2210 * TCC AGG Ser 226	GTC CAG Val * GGG CCC Gly GAC CTG Asp	AAA TTT Lys 21: CAG GTC GIn  * GGC CCG Gly  CAG GTC	CCG Gly 70 * CCG GGC Pro 2220 * TCC AGG Ser 22	* TTC AAG Phe  * GAG CTC Glu  TTC AAG Phe 270 * GGG CCC	TAT ATA Tyr  21  AAC TTG Asn  * TTC AAG Phe	GGG Pro 180 * AAC TTG Asn 223 CTC GAG Leu *	AGC TCG Ser  TAC ATG Tyr  TAT ATA Tyr  2280  TTC AAG	GAC CTG Asp * AAG TTC Lys AGC TCG Ser	ATC TAG Ile 2190 * ACC TGG Thr  AAG TTC Lys  * TGC ACG	ACG TGC Thr CTC GAG Leu 229	GTG CAC Val  * CCT GGA Pro  ACC TGG Thr  GTG CAC	GAG CTC Glu 220 CCC GGG Pro  * GTG CAC Val  ATG TAC	ACC Trp 00 * GTG CAC Val 2250 * GAC CTG Asp	* GAG CTC Glu  * CTG GAC Leu  AAG TTC Lys  GAG CTC	* AGC TCG Ser> GAC CTG Asp> * AGC TCG Ser>

## Fig.31G.

2310 2320 2330 2340 2350 CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys> TGA

ACT

***>

# Fig.32A.

		-	_			•			2.0						
	*	1	.0			20			30			4	0		
ልጥር	GTG.	TCC	CCG	GCG.	CCC		TCC	CCC	CTC.	TCC	CCC.	CTC	CTTC	CTC	TICC.
			GGC												
															Cys>
		•								•					0,0
50			60			7	0			80			90		
*		*	*		*		*	*		*		*	*		*
			GGG												
			CCC												_
Ala	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Ala	Ala	Pro	Thr	Glu	Thr	Gln>
10				110			100				. ^				
10	*	*	4	L10 *		*	120		*	1.	30 *	*	•	140	
CCA	ССТ	GTG	ACA	AAT	ጥጥር	AGT		ጥርጥ	CTT	GAA	AAC	ריזיר	ጥርር	ארא	CTA
			TGT												
															Val>
													-		
	150			10	50		1	L70			180			1	90
*	*		*		*	*		*		*	*		*		*
															CTA
															GAT
TIE	Trp	Thr	Trp	Asn	Pro	Pro	GIU	GIĀ	Ala	Ser	Ser	ASN	Cys	Ser	Leu>
	:	200			210			22	2.0			230			240
*	2	200		*	210		*	22	20	*	,	230		*	240
* TGG		*	AGT	* CAT	*	GGC	* GAC		*	* GAT		*	АТА	* GCT	*
	TAT	* TTT	AGT TCA		* TTT			AAA	* CAA		AAG	* AAA			* CCG
ACC	TAT ATA	* TTT AAA	TCA	GTA	* TTT AAA	CCG	CTG	AAA TTT	* CAA GTT	CTA	AAG TTC	* AAA TTT	TAT	CGA	* CCG
ACC	TAT ATA	* TTT AAA Phe	TCA Ser	GTA	* TTT AAA Phe	CCG	CTG	AAA TTT	* CAA GTT Gln	CTA	AAG TTC	* AAA TTT Lys	TAT	CGA	ccg ggc
ACC	TAT ATA	* TTT AAA Phe	TCA	GTA	* TTT AAA Phe	CCG	CTG	AAA TTT	* CAA GTT	CTA	AAG TTC	* AAA TTT Lys	TAT	CGA	ccg ggc
ACC Trp	TAT ATA Tyr	* TTT AAA Phe	TCA Ser 50	GTA His	* TTT AAA Phe	CCG Gly 260	CTG Asp	AAA TTT Lys	* CAA GTT Gln 270 *	CTA Asp	AAG TTC Lys	* AAA TTT Lys	TAT Ile 80 *	CGA Ala	CCG GGC Pro>
ACC Trp	TAT ATA Tyr * ACT	* TTT AAA Phe 2 CGT	TCA Ser 50 * CGT	GTA His * TCA	TTT AAA Phe	CCG Gly 260 * GAA	CTG Asp	AAA TTT Lys *	* CAA GTT Gln 270 * CTG	CTA Asp	AAG TTC Lys *	* AAA TTT Lys 2	TAT Ile 80 *	CGA Ala *	CCG GGC Pro>
ACC Trp GAA CTT	TAT ATA Tyr  * ACT TGA	TTT AAA Phe 2 CGT GCA	TCA Ser 50 * CGT GCA	GTA His * TCA AGT	TTTT AAA Phe ATA TAT	CCG Gly 260 * GAA CTT	CTG Asp GTA CAT	AAA TTT Lys * CCC GGG	CAA GTT Gln 270 * CTG GAC	CTA Asp AAT TTA	AAG TTC Lys * GAG	AAA TTT Lys 2 AGG	TAT Ile 80 * ATT	CGA Ala * TGT	CCG GGC Pro>
ACC Trp GAA CTT	TAT ATA Tyr  * ACT TGA	TTT AAA Phe 2 CGT GCA	TCA Ser 50 * CGT GCA	GTA His * TCA AGT	TTTT AAA Phe ATA TAT	CCG Gly 260 * GAA CTT	CTG Asp GTA CAT	AAA TTT Lys * CCC GGG	CAA GTT Gln 270 * CTG GAC	CTA Asp AAT TTA	AAG TTC Lys * GAG	AAA TTT Lys 2 AGG	TAT Ile 80 * ATT	CGA Ala * TGT	CCG GGC Pro>
ACC Trp GAA CTT	TAT ATA Tyr  * ACT TGA	TTT AAA Phe 2 CGT GCA	TCA Ser 50 * CGT GCA	GTA His * TCA AGT Ser	TTTT AAA Phe ATA TAT	CCG Gly 260 * GAA CTT Glu	CTG Asp GTA CAT	AAA TTT Lys * CCC GGG	CAA GTT Gln 270 * CTG GAC Leu	CTA Asp AAT TTA	AAG TTC Lys * GAG	AAA TTT Lys 2 AGG	TAT Ile 80 * ATT	CGA Ala * TGT ACA	CCG GGC Pro>
ACC Trp GAA CTT Glu	TAT ATA Tyr  * ACT TGA	TTT AAA Phe 2 CGT GCA	TCA Ser 50 * CGT GCA Arg	GTA His * TCA AGT Ser	TTTT AAA Phe ATA TAT	CCG Gly 260 * GAA CTT Glu	CTG Asp GTA CAT Val	AAA TTT Lys * CCC GGG	CAA GTT Gln 270 * CTG GAC Leu	CTA Asp AAT TTA Asn	AAG TTC Lys * GAG	AAA TTT Lys 2 AGG	TAT Ile 80 * ATT TAA	CGA Ala * TGT ACA	CCG GGC Pro>
GAA CTT Glu 290 *	TAT ATA Tyr * ACT TGA Thr	* TTT AAA Phe 2 CGT GCA Arg	TCA Ser 50 * CGT GCA Arg 300 *	GTA His * TCA AGT Ser	TTTT AAA Phe ATA TAT Ile	CCG Gly 260 * GAA CTT Glu 3	CTG Asp GTA CAT Val 10 *	AAA TTT Lys * CCC GGG Pro	CAA GTT Gln 270 * CTG GAC Leu	AAT TTA ASD  320 * AGT	AAG TTC Lys * GAG CTC	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330	CGA Ala * TGT ACA Cys	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT	TAT ATA Tyr * ACT TGA Thr	* TTT AAA Phe 2 CGT GCA Arg	TCA Ser 50 * CGT GCA Arg 300 * TCC	GTA His  * TCA AGT Ser  CAG GTC	TTTT AAA Phe ATA TAT Ile  * TGT ACA	CCG Gly 260 * GAA CTT Glu 3 AGC TCG	GTA CAT Val  * ACC TGG	AAA TTT Lys * CCC GGG Pro	CAA GTT Gln 270 * CTG GAC Leu GAG CTC	AAT TTA Asn 320 * AGT TCA	AAG TTC Lys GAG CTC	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 * CCT GGA	CGA Ala * TGT ACA Cys	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT	TAT ATA Tyr * ACT TGA Thr	* TTT AAA Phe 2 CGT GCA Arg	TCA Ser 50 * CGT GCA Arg 300 * TCC	GTA His  * TCA AGT Ser  CAG GTC	TTTT AAA Phe ATA TAT Ile  * TGT ACA	CCG Gly 260 * GAA CTT Glu 3 AGC TCG	GTA CAT Val  * ACC TGG	AAA TTT Lys * CCC GGG Pro	CAA GTT Gln 270 * CTG GAC Leu GAG CTC	AAT TTA Asn 320 * AGT TCA	AAG TTC Lys GAG CTC	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 * CCT GGA	CGA Ala * TGT ACA Cys	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT Gln	TAT ATA Tyr  * ACT TGA Thr  GTG CAC Val	* TTT AAA Phe  2 CGT GCA Arg	TCA Ser 50 * CGT GCA Arg 300 * TCC AGG Ser	GTA His  * TCA AGT Ser  CAG GTC Gln	TTTT AAA Phe ATA TAT Ile  * TGT ACA	CCG Gly 260 * GAA CTT Glu 3 AGC TCG	GTA CAT Val  10 * ACC TGG Thr	AAA TTT Lys * CCC GGG Pro * AAT TTA Asn	CAA GTT Gln 270 * CTG GAC Leu GAG CTC	AAT TTA Asn 320 * AGT TCA	* GAG	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 * CCT GGA	CGA Ala  * TGT ACA Cys Cys CSC TCC Ser	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT Gln	TAT ATA Tyr * ACT TGA Thr	* TTT AAA Phe  2 CGT GCA Arg	TCA Ser 50 * CGT GCA Arg 300 * TCC AGG Ser	GTA His  * TCA AGT Ser  CAG GTC	TTTT AAA Phe ATA TAT Ile  * TGT ACA	CCG Gly 260 * GAA CTT Glu 3 AGC TCG	GTA CAT Val  * ACC TGG	AAA TTT Lys * CCC GGG Pro * AAT TTA Asn	CAA GTT Gln 270 * CTG GAC Leu GAG CTC	AAT TTA Asn 320 * AGT TCA	AAG TTC Lys GAG CTC	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 * CCT GGA	CGA Ala * TGT ACA Cys	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT Gln	TAT ATA Tyr * ACT TGA Thr GTG CAC Val	TTTT AAA Phe  2 CGT GCA Arg  * GGG CCC Gly	TCA Ser 50 * CGT GCA Arg 300 * TCC AGG Ser	GTA His  * TCA AGT Ser  CAG GTC Gln 350 *	TTTT AAA Phe ATA TAT Ile TGT ACA Cys	CCG Gly 260 * GAA CTT Glu 3 AGC TCG Ser	GTA CAT Val  10 * ACC TGG Thr 360	AAA TTT Lys * CCC GGG Pro * AAT TTA Asn	CAA GTT Gln 270 * CTG GAC Leu GAG CTC Glu	AAT TTA ASN 320 * AGT TCA Ser	AAG TTC Lys  * GAG CTC Glu  GAG TTC	AAA TTT Lys  2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 * CCT GGA	CGA Ala  * TGT ACA Cys TCG	CCG GGC Pro> CTG GAC Leu>  ATT TAA TILe>
GAA CTT Glu 290 * CAA GTT Gln 3	TAT ATA Tyr  * ACT TGA Thr  GTG CAC Val  40 * GTT	* TTTT AAA Phe  2 CGT GCA Arg  * GGG CCC Gly  * GAA	TCA Ser 50 * CGT GCA Arg 300 * TCC AGG Ser	TCA AGT Ser  CAG GTC Gln 350 *	TTTT AAA Phe ATA TAT Ile TGT ACA Cys	CCG Gly 260 * GAA CTT Glu 3 AGC TCG Ser	GTA CAT Val  ACC TGG Thr  360 *	AAA TTT Lys  * CCC GGG Pro  * AAT TTA Asn	CAA GTT Gln 270 * CTG GAC Leu GAG CTC Glu *	AAT TTA ASN 320 * AGT TCA Ser 3	AAG TTC Lys GAG CTC Glu GAG CTC Glu 70 *	AAA TTT Lys 2 AGG TCC Arg	TAT Ile 80 * ATT TAA Ile 330 CCT GGA	CGA Ala  * TGT ACA Cys	CCG GGC Pro> CTG GAC Leu>
GAA CTT Glu 290 * CAA GTT Gln 3 TTG AAC	TAT ATA TYT  * ACT TGA Thr  GTG CAC Val  40 * GTT CAA	* TTTT AAA Phe  2 CGT GCA Arg  * GGG CCC Gly  GAA CTT	TCA Ser 50 * CGT GCA Arg 300 * TCC AGG Ser	GTA His  TCA AGT Ser  CAG GTC Gln 350  TGC ACG	TTTT AAA Phe ATA TAT Ile  * TGT ACA Cys	CCG Gly 260 * GAA CTT Glu 3 AGC TCG Ser * TCA AGT	GTA CAT Val  10 * ACC TGG Thr  360 * CCC	AAA TTT Lys  * CCC GGG Pro  * AAT TTA Asn	CAA GTT Gln 270 * CTG GAC Leu GAG CTC Glu * GAA CTT	AAT TTA Asn 320 * AGT TCA Ser 3	* GAG CTC GAG CTC GAT	AAA TTT Lys 2 AGG TCC ATG ATG Lys CCT GGA	TAT Ile 80 * ATT TAA Ile 330 * CCT GGG	CGA Ala  * TGT ACA Cys Cys 380 * TCT AGA CA CA AGA CA AGA CA CA AGA CA	CCG GGC Pro> CTG GAC Leu>  ATT TAA TIle>

# Fig.32B.

							$\cup$								
	390			4	00			110			420			4:	30
*	*		*		*	*		*		*	*		*		*
GTG	ACT	GAG	CTT	CAA	TGC	ATT	TGG	CAC	AAC	CTG	AGC	TAC	ATG	DAA	ጥርጥ
CAC	TGA	CTC	GAA	GTT	ACG	TAA	ACC	GTG	TTG	GAC	TCG	ATG	TAC	חיויי	7C7
Val	Thr	Glu	Leu	Gln	Cvs	Ile	Trp	His	Asn	Leu	Ser	Tyr	Mat	Tyc	Cys>
					•						001	+ 7 -	Mec	пуs	Cys>
		140			450			46	50		,	170			400
*		*		*	*		*	- `	*	*	-	*		•	480
TCT	TGG	CTC	ССТ	GGA	AGG	ААТ	ACC	AGT	CCC	GAC	A C T	220	መአመ	" 3 O M	
AGA	ACC	GAG	GGA	CCT	TCC	ጥጥል	TGG	TCA	GGG	CTG	WC I	MMC	IWI	ACT	CTC
Ser	Trp	Leu	Pro	Glv	Ανα	Acn	Thr	Cox	D~0	700	Mb	116	ATA	TGA	GAG Leu>
	•			1	•••	7.511	1111	Ser	FIO	ASD	TIII	ASII	ıyr	Thr	Leu>
		49	90			500			510			E 7	. ^		
	*		*	*	•	*		*	*		•	52	20		
TAC	TAT	TGG	CAC	AGA	AGC	СТС	CAA	מממ		CAT	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	mam	~		
ATG	ATA	ACC	GTG	ጥርጥ	TCG	CAC	Cut	WWW.	W Y Y	GTA	CAA	161	GAA	AAC	ATC
Tvr	Tvr	Trn	His	Ara	Ser	Lau	Clu	TAG	Tlo	GIA	GIT.	ACA	CTT	TTG	TAG Ile>
- 4 -	-1-			**** 9	561	Dea	GIU	Lys	116	HIS	GIN	Cys	GLU	Asn	Ile>
530			540			5	50			60					
*		*	*		*	J.	*	•	-	,60		_	570		
TTT	AGA	GAA	GGC	ממים	ጥልሮ	- Trippy		mcm	mcc.	TTT	<b>~~</b>	×	*		*
AAA	TCT	Cuu	CCG	CTT	ATC	777	CCX	101	100	7.1.1.	GAT.	CTG	ACC	AAA	GTG
Phe	Ara	Glu	Gly	Gla	W	Dho	CLA	ACA	AGG	AAA	CTA	GAC	TGG	TTT	CAC
	•••	Gru	Gry	GIII	TAT	Pile	GTA	Cys	ser	rne	Asp	Leu	Thr	Lys	Val>
														_	
58		*		590		*	600			61				520	
58	30 *	*	5	590		*	600 *		*	61	LO *	*	•	520	
58 AAG	30 * GAT	* TCC	AGT	590 * TTT	GAA	* CAA	600 * CAC	AGT	* GTC	61 CAA	LO * ATA	* ATG	GTC	520 * AAG	GAT
58 AAG TTC	30 * GAT CTA	* TCC AGG	AGT TCA	590 * TTT AAA	GAA CTT	* CAA GTT	600 * CAC GTG	AGT TCA	* GTC CAG	61 CAA GTT	LO * ATA TAT	* ATG TAC	GTC CAG	AAG	GAT CTA
58 AAG TTC	30 * GAT CTA	* TCC AGG	AGT TCA	590 * TTT AAA	GAA CTT	* CAA GTT	600 * CAC GTG	AGT TCA	* GTC CAG	61 CAA GTT	LO * ATA TAT	* ATG TAC	GTC CAG	AAG	GAT
58 AAG TTC	GAT CTA Asp	* TCC AGG	AGT TCA	TTT AAA Phe	GAA CTT Glu	* CAA GTT	600 * CAC GTG His	AGT TCA Ser	* GTC CAG	61 CAA GTT	ATA TAT	* ATG TAC	GTC CAG	AAG TTC Lys	GAT CTA Asp>
58 AAG TTC	30 * GAT CTA	* TCC AGG	AGT TCA	590 * TTT AAA	GAA CTT Glu	* CAA GTT	600 * CAC GTG His	AGT TCA Ser	* GTC CAG	61 CAA GTT	LO * ATA TAT	* ATG TAC	GTC CAG	AAG TTC Lys	GAT CTA
AAG TTC Lys	GAT CTA Asp	* TCC AGG Ser	AGT TCA Ser	590 * TTT AAA Phe	GAA CTT Glu 10	* CAA GTT Gln *	600 * CAC GTG His	AGT TCA Ser 550	* GTC CAG Val	61 CAA GTT Gln	ATA TAT Ile	* ATG TAC Met	GTC CAG Val	AAG TTC Lys	GAT CTA Asp>
AAG TTC Lys *	GAT CTA Asp 630	* TCC AGG Ser	AGT TCA Ser *	590 * TTT AAA Phe 64	GAA CTT Glu 10 *	* CAA GTT Gln * CCA	600 * CAC GTG His	AGT TCA Ser 550 *	* GTC CAG Val	CAA GTT Gln *	ATA TAT Ile 660 *	* ATG TAC Met	GTC CAG Val	AAG TTC Lys 67	GAT CTA Asp>
AAG TTC Lys * AAT	GAT CTA Asp 630 * GCA CGT	* TCC AGG Ser GGA CCT	AGT TCA Ser * AAA TTT	590 * TTT AAA Phe 64 ATT TAA	GAA CTT Glu 10 * AAA TTT	* CAA GTT Gln  * CCA GGT	600 * CAC GTG His	AGT TCA Ser 550 * TTC	* GTC CAG Val AAT	CAA GTT Gln * ATA	ATA TAT Ile 660 * GTG CAC	* ATG TAC Met	GTC CAG Val * TTA	AAG TTC Lys 67	GAT CTA Asp>  10 * TCC AGG
AAG TTC Lys * AAT	GAT CTA Asp 630 * GCA CGT	* TCC AGG Ser GGA CCT	AGT TCA Ser * AAA TTT	590 * TTT AAA Phe 64 ATT TAA	GAA CTT Glu 10 * AAA TTT	* CAA GTT Gln  * CCA GGT	600 * CAC GTG His	AGT TCA Ser 550 * TTC	* GTC CAG Val AAT	CAA GTT Gln * ATA	ATA TAT Ile 660 * GTG CAC	* ATG TAC Met	GTC CAG Val * TTA	AAG TTC Lys 67	GAT CTA Asp>
AAG TTC Lys * AAT	GAT CTA Asp 630 * GCA CGT	* TCC AGG Ser GGA CCT Gly	AGT TCA Ser * AAA TTT	590 * TTT AAA Phe 64 ATT TAA	GAA CTT Glu 10 * AAA TTT Lys	* CAA GTT Gln  * CCA GGT	600 * CAC GTG His TCC AGG	AGT TCA Ser 550 * TTC AAG	* GTC CAG Val AAT TTA Asn	CAA GTT Gln * ATA	ATA TAT Ile 660 * GTG CAC Val	* ATG TAC Met CCT GGA Pro	GTC CAG Val * TTA	AAG TTC Lys 67	GAT CTA Asp>  O * TCC AGG Ser>
AAG TTC Lys * AAT	GAT CTA Asp 630 * GCA CGT	* TCC AGG Ser GGA CCT	AGT TCA Ser * AAA TTT	590 * TTT AAA Phe 64 ATT TAA	GAA CTT Glu 10 * AAA TTT	* CAA GTT Gln  * CCA GGT	600 * CAC GTG His TCC AGG	AGT TCA Ser 550 * TTC	* GTC CAG Val AAT TTA Asn	CAA GTT Gln * ATA	ATA TAT Ile 660 * GTG CAC Val	* ATG TAC Met	GTC CAG Val * TTA	AAG TTC Lys 67	GAT CTA Asp>  10 * TCC AGG
AAG TTC Lys * AAT TTA Asn	GAT CTA Asp 630 * GCA CGT Ala	* TCC AGG Ser GGA CCT Gly 680 *	AGT TCA Ser * AAA TTT Lys	TTT AAA Phe 64 ATT TAA Ile	GAA CTT Glu 10 * AAA TTT Lys 690 *	* CAA GTT Gln  * CCA GGT Pro	600  * CAC GTG His  TCC AGG Ser	AGT TCA Ser 550 * TTC AAG Phe	* GTC CAG Val AAT TTA Asn	CAA GTT Gln * ATA TAT Ile	ATA TAT Ile 660 * GTG CAC Val	* ATG TAC Met CCT GGA Pro	GTC CAG Val * TTA AAT Leu	AAG TTC Lys 67 ACT TGA Thr	GAT CTA Asp> 70 * TCC AGG Ser> 720
AAG TTC Lys * AAT TTA Asn	GTG	* TCC AGG Ser GGA CCT Gly 80 * AAA	AGT TCA Ser * AAA TTT Lys	590  TTT AAA Phe 64 ATT TAA Ile *	GAA CTT Glu 10 * AAA TTT Lys 690 *	* CAA GTT Gln  * CCA GGT Pro	600  * CAC GTG His  TCC AGG Ser  *	AGT TCA Ser TTC AAG Phe	* GTC CAG Val AAT TTA Asn 0 *	CAA GTT Gln * ATA TAT Ile	ATA TAT Ile 660  GTG CAC Val	ATG TAC Met CCT GGA Pro 10 *	GTC CAG Val * TTA AAT Leu	AAG TTC Lys 67 ACT TGA Thr	GAT CTA Asp> 70 * TCC AGG Ser> 720 *
AAG TTC Lys * AAT TTA Asn * CGT GCA	GTG	TCC AGG Ser  GGA CCT Gly  880 * AAA TTT	AGT TCA Ser * AAA TTT Lys	TTT AAA Phe 64 ATT TAA Ile * GAT CTA	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA	* CAA GTT Gln  * CCA GGT Pro	CAC GTG His TCC AGG Ser  * CAT	AGT TCA Ser TTC AAG Phe 70	* GTC CAG Val AAT TTA Asn 0 * AAA	CAA GTT Gln * ATA TAT Ile *	ATA TAT Ile 660 * GTG CAC Val CTC GAG	ATG TAC Met  CCT GGA Pro 10 * TCC AGG	GTC CAG Val * TTA AAT Leu	AAG TTC Lys 67 ACT TGA Thr * CAC	GAT CTA Asp> 70 * TCC AGG Ser> 720 * AAT TTA
AAG TTC Lys * AAT TTA Asn * CGT GCA	GTG	TCC AGG Ser  GGA CCT Gly  880 * AAA TTT	AGT TCA Ser * AAA TTT Lys	TTT AAA Phe 64 ATT TAA Ile * GAT CTA	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA	* CAA GTT Gln  * CCA GGT Pro	CAC GTG His TCC AGG Ser  * CAT	AGT TCA Ser TTC AAG Phe 70	* GTC CAG Val AAT TTA Asn 0 * AAA	CAA GTT Gln * ATA TAT Ile *	ATA TAT Ile 660 * GTG CAC Val CTC GAG	ATG TAC Met  CCT GGA Pro 10 * TCC AGG	GTC CAG Val * TTA AAT Leu	AAG TTC Lys 67 ACT TGA Thr * CAC	GAT CTA Asp> 70 * TCC AGG Ser> 720 *
AAG TTC Lys * AAT TTA Asn * CGT GCA	GTG	* TCC AGG Ser  GGA CCT Gly  80 * AAA TTT	AGT TCA Ser * AAA TTT Lys CCT GGA Pro	TTT AAA Phe 64 ATT TAA Ile * GAT CTA	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA Pro	* CAA GTT Gln  * CCA GGT Pro	CAC GTG His TCC AGG Ser  * CAT	AGT TCA Ser TTC AAG Phe 70	* GTC CAG Val AAT TTA ASN 0 * AAA TTT Lys	CAA GTT Gln * ATA TAT Ile *	ATA TAT Ile 660 * GTG CAC Val CTC GAG	ATG TAC Met  CCT GGA Pro  10 * TCC AGG Ser	GTC CAG Val * TTA AAT Leu TTC AAG Phe	AAG TTC Lys 67 ACT TGA Thr * CAC	GAT CTA Asp> 70 * TCC AGG Ser> 720 * AAT
AAG TTC Lys * AAT TTA Asn * CGT GCA	GTG	TCC AGG Ser  GGA CCT Gly  880 * AAA TTT	AGT TCA Ser * AAA TTT Lys CCT GGA Pro	TTT AAA Phe 64 ATT TAA Ile * GAT CTA	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA Pro	* CAA GTT Gln  * CCA GGT Pro	CAC GTG His TCC AGG Ser  * CAT	AGT TCA Ser TTC AAG Phe 70	* GTC CAG Val AAT TTA Asn 0 * AAA	CAA GTT Gln * ATA TAT Ile *	ATA TAT Ile 660 * GTG CAC Val CTC GAG	ATG TAC Met  CCT GGA Pro 10 * TCC AGG	GTC CAG Val * TTA AAT Leu TTC AAG Phe	AAG TTC Lys 67 ACT TGA Thr * CAC GTG His	GAT CTA Asp> 70 * TCC AGG Ser> 720 * AAT
AAG TTC Lys * AAT TTA Asn * CGT GCA Arg	GTG CAC Val	* TCC AGG Ser GGA CCT Gly 80 * AAA TTT Lys	AGT TCA Ser * AAA TTT Lys CCT GGA Pro	TTT AAA Phe 64 ATT TAA Ile * GAT CTA Asp	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA Pro	* CAA GTT Gln  * CCA GGT Pro  CCA GGT Pro  740 *	CAC GTG His TCC AGG Ser * CAT GTA His	AGT TCA Ser TTC AAG Phe 70 ATT TAA Ile	* GTC CAG Val  AAT TTA Asn 0 * AAA TTT Lys 750 *	CAA GTT Gln * ATA TAT Ile * AAC TTG Asn	ATA TAT Ile 660 * GTG CAC Val  CTC GAG Leu	ATG TAC Met  CCT GGA Pro  10 * TCC AGG Ser	GTC CAG Val  * TTA AAT Leu  TTC AAG Phe	AAG TTC Lys 67 ACT TGA Thr * CAC GTG His	GAT CTA Asp>  70 * TCC AGG Ser>  720 * AAT TTA Asn>
AAG TTC Lys * AAT TTA Asn * CGT GCA Arg	GTG CAC Val	TCC AGG Ser  GGA CCT Gly  80  AAA TTT Lys  73	AGT TCA Ser * AAA TTT Lys CCT GGA Pro	TTTT AAA Phe 64 ATT TAA Ile * GAT CTA Asp	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA Pro	* CAA GTT Gln  * CCA GGT Pro  CCA GGT Pro  * TGG	CAC GTG His TCC AGG Ser  * CAT GTA His	AGT TCA Ser 550 * TTC AAG Phe 70 ATT TAA Ile	* GTC CAG Val  AAT TTA ASN 0 * AAA TTT Lys 750 * CCA	CAA GTT Gln * ATA TAT Ile * AAC TTG Asn	ATA TAT Ile 660 * GTG CAC Val  CTC GAG Leu	ATG TAC Met  CCT GGA Pro  10 * TCC AGG Ser	GTC CAG Val  * TTA AAT Leu  TTC AAG Phe	AAG TTC Lys 67 ACT TGA Thr * CAC GTG His	GAT CTA Asp> 70 * TCC AGG Ser> 720 * AAT TTA Asn>
AAG TTC Lys * AAT TTA ASN * CGT GCA Arg	GAT CTA ASP 630 * GCA CGT Ala GTG CAC Val * GAC CTG	TCC AGG Ser  GGA CCT Gly  80 * AAA TTT Lys  73 CTA GAT	AGT TCA Ser * AAA TTT Lys CCT GGA Pro	TTTT AAA Phe 64 ATT TAA Ile * GAT CTA Asp * GTG CAC	GAA CTT Glu 10 * AAA TTT Lys 690 * CCT GGA Pro	* CAA GTT Gln  * CCA GGT Pro  CCA GGT Pro  TGG ACC	CAC GTG His TCC AGG Ser  * CAT GTA His	AGT TCA Ser 50 * TTC AAG Phe 70 ATT TAA Ile	* GTC CAG Val  AAT TTA ASN 0 * AAA TTT Lys 750 * CCA GGT	CAA GTT Gln  * ATA TAT Ile  * AAC TTG Asn	ATA TAT Ile 660 * GTG CAC Val  CTC GAG Leu AAT	ATG TAC Met  CCT GGA Pro  10 * TCC AGG Ser  76	GTC CAG Val  * TTA AAT Leu  TTC AAG Phe  * ATT	AAG TTC Lys 67 ACT TGA Thr * CAC GTG His	GAT CTA Asp>  70 * TCC AGG Ser>  720 * AAT TTA Asn>

# Fig.32C.

770			200												
770 *		_	780			79	90		8	300			810		
	Cmx	mmm			*		*	*		*		*	*		*
ACC	CIM	1.1.1	TAT	GAA	GTA	GAA	GTC	AAT	AAC	AGC	CAA	ACT	GAG	ACA	CAT
Cve	Lau	Pho	MIA	CIT	CAT	CTT	CAG	TTA	TTG	TCG	GTT	TGA	CTC	TGT	GTA
Cys	Deu	FIIE	IVI	GIU	vaı	GIU	vaı	Asn	Asn	Ser	Gin	Thr	GIu	Thr	His>
82	20		٤	330			840			85	٠ <u>٠</u>			360	
	*	*		*		*	*		*	0.	*	*	•	*	
AAT	GTT	TTC	TAC	GTC	CAA	GAG	GCT	AAA	TGT	GAG	AAT	CCA	GAA	ተተ	GAG
TTA	CAA	AAG	ATG	CAG	GTT	CTC	CGA	TTT	ACA	CTC	TTA	GGT	CTT	AAA	CTC
Asn	Val	Phe	Tyr	Val	Gln	Glu	Ala	Lys	Cys	Glu	Asn	Pro	Glu	Phe	Glu>
															<del>_</del>
	870			88	30		8	390			900			9:	LO
*	*		*		*	*		*		*	*		*		*
AGA	AAT	GTG	GAG	AAT	ACA	TCT	TGT	TTC	ATG	GTC	CCT	GGT	GTT	CTT	CCT
TCT	TTA	CAC	CTC	TTA	TGT	AGA	ACA	AAG	TAC	CAG	GGA	CCA	CAA	GAA	GGA
Arg	Asn	Val	Glu	Asn	Thr	Ser	Cys	Phe	Met	Val	Pro	Gly	Val	Leu	Pro>
	•	920			930			9.4	10			950			0.50
*	•	*		*	*		*	٠,	*	*	•	*		*	960
GAT	ACT	TTG	AAC	ACA	GTC	AGA	АТА	AGA	GTC	AAA	ACA	ТАА	AAC	ጥጥል	
										TTT					
Asp	Thr	Leu	Asn	Thr	Val	Arg	Ile	Arg	Val	Lys	Thr	Asn	Lvs	Leu	Cys>
								_		_			-		
		97	70		9	980			990			100	00		
	*		*	*		*		*	*		*		*	*	
		GAT	* GAC		CTC	* TGG		AAT	* TGG	AGC		GAA	* ATG	AGT	
ATA	CTC	GAT CTA	* GAC CTG	TTT	CTC GAG	* TGG ACC	TCA	AAT TTA	* TGG ACC	TCG	GTT	GAA CTT	* ATG TAC	AGT TCA	TAT
ATA	CTC	GAT CTA	* GAC CTG	TTT	CTC GAG	* TGG ACC	TCA	AAT TTA	* TGG ACC	TCG	GTT	GAA CTT	* ATG TAC	AGT TCA	
ATA	CTC	GAT CTA Asp	* GAC CTG Asp	TTT	CTC GAG	* TGG ACC Trp	TCA Ser	AAT TTA	* TGG ACC Trp	TCG Ser	GTT	GAA CTT Glu	* ATG TAC Met	AGT TCA	TAT
ATA Tyr	CTC	GAT CTA Asp	* GAC CTG	TTT	CTC GAG	* TGG ACC	TCA Ser	AAT TTA	* TGG ACC Trp	TCG	GTT	GAA CTT Glu	* ATG TAC	AGT TCA	TAT
ATA Tyr 1010	CTC	GAT CTA Asp	* GAC CTG Asp	TTT Lys	CTC GAG Leu	* TGG ACC Trp	TCA Ser 30	AAT TTA Asn	TGG ACC Trp	TCG Ser 040	GTT Gln	GAA CTT Glu	* ATG TAC Met 1050	AGT TCA Ser	TAT Ile>
ATA Tyr 1010 * GGT CCA	CTC Glu AAG TTC	GAT CTA Asp * AAG TTC	GAC CTG Asp L020 * CGC GCG	TTT Lys AAT TTA	CTC GAG Leu * TCC AGG	TGG ACC Trp 103	TCA Ser 30 * GGC CCG	AAT TTA Asn * GCG CGC	TGG ACC Trp 10 CCT GGA	TCG Ser 040 * AGT TCA	GTT Gln GGT CCA	GAA CTT Glu * GGA CCT	* ATG TAC Met 1050 * GGT CCA	AGT TCA Ser GGC CCG	TAT Ile>  * CGG GCC
ATA Tyr 1010 * GGT CCA	CTC Glu AAG TTC	GAT CTA Asp * AAG TTC	GAC CTG Asp L020 * CGC GCG	TTT Lys AAT TTA	CTC GAG Leu * TCC AGG	TGG ACC Trp 103	TCA Ser 30 * GGC CCG	AAT TTA Asn * GCG CGC	TGG ACC Trp 10 CCT GGA	TCG Ser 040 * AGT TCA	GTT Gln GGT CCA	GAA CTT Glu * GGA CCT	* ATG TAC Met 1050 * GGT CCA	AGT TCA Ser GGC CCG	TAT Ile>  * CGG
ATA Tyr 1010 * GGT CCA Gly	CTC Glu AAG TTC Lys	GAT CTA Asp * AAG TTC	GAC CTG Asp L020 * CGC GCG Arg	TTT Lys AAT TTA Asn	CTC GAG Leu * TCC AGG	* TGG ACC Trp 103 ACA TGT Thr	TCA Ser 30 * GGC CCG Gly	AAT TTA Asn * GCG CGC	TGG ACC Trp 10 CCT GGA	TCG Ser 040 * AGT TCA Ser	GTT Gln GGT CCA Gly	GAA CTT Glu * GGA CCT	ATG TAC Met 1050 * GGT CCA Gly	AGT TCA Ser GGC CCG Gly	TAT Ile>  * CGG GCC
ATA Tyr 1010 * GGT CCA	CTC Glu AAG TTC Lys	GAT CTA Asp * AAG TTC	GAC CTG Asp L020 * CGC GCG Arg	TTT Lys AAT TTA Asn	CTC GAG Leu * TCC AGG	* TGG ACC Trp 103 ACA TGT Thr	TCA Ser 30 * GGC CCG	AAT TTA Asn * GCG CGC	TGG ACC Trp 10 CCT GGA	TCG Ser 040 * AGT TCA	GTT Gln GGT CCA Gly	GAA CTT Glu * GGA CCT	ATG TAC Met 1050 * GGT CCA Gly	AGT TCA Ser GGC CCG	TAT Ile>  * CGG GCC
ATA Tyr  1010  GGT CCA Gly	AAG TTC Lys	GAT CTA Asp * AAG TTC Lys	GAC CTG Asp L020 * CGC GCG Arg	TTT Lys AAT TTA Asn 070	CTC GAG Leu * TCC AGG Ser	* TGG ACC Trp 103 ACA TGT Thr	TCA Ser 80 * GGC CCG Gly 1080	AAT TTA Asn * GCG CGC Ala	TGG ACC Trp  10 CCT GGA Pro	TCG Ser 040 * AGT TCA Ser	GTT Gln GGT CCA Gly	GAA CTT Glu * GGA CCT Gly	* ATG TAC Met 1050 * GGT CCA Gly	AGT TCA Ser GGC CCG Gly 100	TAT Ile>  * CGG GCC Arg>
ATA Tyr  1010 * GGT CCA Gly  100	AAG TTC Lys 60 *	GAT CTA Asp * AAG TTC Lys	GAC CTG Asp L020 * CGC GCG Arg	AAT TTA ASn 070 * GGG	CTC GAG Leu * TCC AGG Ser	TGG ACC Trp 103 ACA TGT Thr	TCA Ser 30 * GGC CCG Gly 1080 *	AAT TTA Asn * GCG CGC Ala	TGG ACC Trp	TCG Ser 040 * AGT TCA Ser 10	GTT Gln GGT CCA Gly 90 *	GAA CTT Glu * GGA CCT Gly	* ATG TAC Met 1050 * GGT CCA Gly 1	AGT TCA Ser GGC CCG Gly 100 *	TAT Ile>  * CGG GCC Arg>
ATA Tyr  1010 * GGT CCA Gly 100 CCC GGG	AAG TTC Lys 60 * GCA CGT	GAT CTA Asp * AAG TTC Lys AGC TCG	GAC CTG Asp L020 * CGC GCG Arg  TCT AGA	AAT TTA ASN 070 * GGG CCC	CTC GAG Leu * TCC AGG Ser AAC TTG	TGG ACC Trp 103 ACA TGT Thr ATG TAC	TCA Ser 30 * GGC CCG Gly 1080 * AAG	AAT TTA Asn GCG CGC Ala GTC CAG	TGG ACC Trp  10 CCT GGA Pro  TTG AAC	TCG Ser 040 * AGT TCA Ser 100 CAG GTC	GTT Gln GGT CCA Gly 90 * GAG CTC	GAA CTT Glu * GGA CCT Gly CCC GGG	ATG TAC Met  1050  GGT CCA Gly  ACC TGG	AGT TCA Ser GGC CCG Gly 100 * TGC ACG	TAT Ile>  * CGG GCC Arg> GTC CAG
ATA Tyr  1010 * GGT CCA Gly 100 CCC GGG	AAG TTC Lys 60 * GCA CGT	GAT CTA Asp * AAG TTC Lys AGC TCG	GAC CTG Asp L020 * CGC GCG Arg  TCT AGA	AAT TTA ASN 070 * GGG CCC	CTC GAG Leu * TCC AGG Ser AAC TTG	TGG ACC Trp 103 ACA TGT Thr ATG TAC	TCA Ser 30 * GGC CCG Gly 1080 * AAG	AAT TTA Asn GCG CGC Ala GTC CAG	TGG ACC Trp  10 CCT GGA Pro  TTG AAC	TCG Ser 040 * AGT TCA Ser 100 CAG GTC	GTT Gln GGT CCA Gly 90 * GAG CTC	GAA CTT Glu * GGA CCT Gly CCC GGG	ATG TAC Met  1050  GGT CCA Gly  ACC TGG	AGT TCA Ser GGC CCG Gly 100 * TGC ACG	TAT Ile>  * CGG GCC Arg>
ATA Tyr  1010  GGT CCA Gly  100  CCC GGG	AAG TTC Lys 60 * GCA CGT	GAT CTA Asp * AAG TTC Lys AGC TCG	GAC CTG Asp L020 * CGC GCG Arg  TCT AGA	AAT TTA ASN 070 * GGG CCC	CTC GAG Leu * TCC AGG Ser AAC TTG Asn	TGG ACC Trp 103 ACA TGT Thr ATG TAC	TCA Ser 30 * GGC CCG Gly 1080 * AAG TTC Lys	AAT TTA Asn GCG CGC Ala GTC CAG	TGG ACC Trp  10 CCT GGA Pro  TTG AAC	TCG Ser 040 * AGT TCA Ser 100 CAG GTC GIn	GTT Gln GGT CCA Gly 90 * GAG CTC	GAA CTT Glu * GGA CCT Gly CCC GGG	ATG TAC Met  1050  GGT CCA Gly  ACC TGG	AGT TCA Ser GGC CCG Gly 100 * TGC ACG Cys	TAT Ile>  * CGG GCC Arg> GTC CAG
ATA Tyr  1010 * GGT CCA Gly 10 CCC GGG Pro	AAG TTC Lys 60 * GCA CGT Ala 1110 *	GAT CTA Asp * AAG TTC Lys AGC TCG Ser	GAC CTG ASP L020 * CGC GCG ATG TCT AGA Ser	AAT TTA ASN O70 * GGG CCC Gly	CTC GAG Leu * TCC AGG Ser AAC TTG Asn	TGG ACC Trp 103 ACA TGT Thr * ATG TAC Met	TCA Ser 30 * GGC CCG Gly 1080 * AAG TTC Lys	AAT TTA Asn  * GCG CGC Ala  GTC CAG Val  130 *	TGG ACC Trp  10 CCT GGA Pro  * TTG AAC Leu	TCG Ser 040 * AGT TCA Ser 100 CAG GTC Gln	GTT Gln GGT CCA Gly 90 * GAG CTC Glu	GAA CTT Glu * GGA CCT Gly * CCC GGG	* ATG TAC Met  1050 * GGT CCA Gly  1 ACC TGG Thr	AGT TCA Ser GGC CCG Gly 100 * TGC ACG Cys	TAT Ile>  * CGG GCC Arg>  GTC CAG Val> 50 *
ATA Tyr  1010 * GGT CCA Gly 10 CCC GGG Pro * TCC	AAG TTC Lys 60 * GCA CGT Ala 1110 *	GAT CTA Asp * AAG TTC Lys AGC TCG Ser	GAC CTG Asp L020 * CGC GCG Arg TCT AGA Ser *	AAT TTA ASN O70 * GGG CCC Gly 11:	CTC GAG Leu * TCC AGG Ser AAC TTG Asn	TGG ACC Trp  103  ACA TGT Thr  ATG TAC Met	TCA Ser 30 * GGC CCG Gly 1080 * AAG TTC Lys	AAT TTA Asn  * GCG CGC Ala  GTC CAG Val  130  * TGC	TGG ACC Trp  10 CCT GGA Pro  TTG AAC Leu	TCG Ser 040 * AGT TCA Ser 100 CAG GTC GIn	GTT Gln GGT CCA Gly 90 * GAG CTC Glu 1140 *	GAA CTT Glu * GGA CCT Gly CCC GGG Pro	ATG TAC Met  1050  GGT CCA Gly  ACC TGG Thr	AGT TCA Ser GGC CCG Gly 100 * TGC ACG Cys	TAT Ile>  * CGG GCC Arg>  GTC CAG Val> 50 * CCC
ATA Tyr  1010  * GGT CCA Gly  10  CCC GGG Pro  * TCC AGG	AAG TTC Lys 60 * GCA CGT Ala 1110 * GAC CTG	GAT CTA Asp * AAG TTC Lys * AGC TCG Ser	GAC CTG Asp L020 * CGC GCG Arg TCT AGA Ser ATG TAC	AAT TTA ASN O70 * GGG CCC Gly 11: AGC TCG	CTC GAG Leu * TCC AGG Ser AAC TTG Asn	* TGG ACC Trp 103 ACA TGT Thr * ATG TAC Met  * TCT AGA	TCA Ser 30 * GGC CCG Gly 1080 * AAG TTC Lys	AAT TTA Asn  * GCG CGC Ala  GTC CAG Val  130  * TGC ACG	TGG ACC Trp  10 CCT GGA Pro  * TTG AAC Leu GAG CTC	TCG Ser 040 * AGT TCA Ser 10: CAG GTC GIn * TGG ACC	GTT Gln GGT CCA Gly 90 * GAG CTC Glu 1140 * AAG	GAA CTT Glu * GGA CCT Gly CCC GGG Pro	* ATG TAC Met  1050  * GGT CCA Gly  1 ACC TGG Thr  * AAT	AGT TCA Ser GGC CCG Gly 100 * TGC ACG Cys 11	TAT Ile>  * CGG GCC Arg>  GTC CAG Val> 50 * CCC GGG
ATA Tyr  1010  * GGT CCA Gly  10  CCC GGG Pro  * TCC AGG	AAG TTC Lys 60 * GCA CGT Ala 1110 * GAC CTG	GAT CTA Asp * AAG TTC Lys * AGC TCG Ser	GAC CTG Asp L020 * CGC GCG Arg TCT AGA Ser ATG TAC	AAT TTA ASN O70 * GGG CCC Gly 11: AGC TCG	CTC GAG Leu * TCC AGG Ser AAC TTG Asn	* TGG ACC Trp 103 ACA TGT Thr * ATG TAC Met  * TCT AGA	TCA Ser 30 * GGC CCG Gly 1080 * AAG TTC Lys	AAT TTA Asn  * GCG CGC Ala  GTC CAG Val  130  * TGC ACG	TGG ACC Trp  10 CCT GGA Pro  * TTG AAC Leu GAG CTC	TCG Ser 040 * AGT TCA Ser 10: CAG GTC GIn * TGG ACC	GTT Gln GGT CCA Gly 90 * GAG CTC Glu 1140 * AAG	GAA CTT Glu * GGA CCT Gly CCC GGG Pro	* ATG TAC Met  1050  * GGT CCA Gly  1 ACC TGG Thr  * AAT	AGT TCA Ser GGC CCG Gly 100 * TGC ACG Cys 11	TAT Ile>  * CGG GCC Arg>  GTC CAG Val> 50 * CCC

# Fig.32D.

	11	.60		1	170			118	0		11	.90		1	.200
*		*		*	*		*		*	*		*		*	*
ACC	AAT	TGC	AGC	ACC	GAG	CTC	CGC	CTG	TTG	TAC	CAG	CTG	GTT	TTT	CTG
							GCG								
Thr	Asn	Cys	Ser	Thr	Glu	Leu	Arg	Leu	Leu	Tyr	Gln	Leu	Val	Phe	Leu>
	*	121	.0		12	20		_ 1	.230			124	<b>*</b>		
כידיכ	ጥሮር	GAA	GCC.	ראכ	ACC.	m∩m ·	ATC		CNC	አአር	7 7 7 C	CCA		~~~	000
							TAG								
															Gly>
						C			GLU			GLY	Gry	AIG	GIA>
1250		3	260			127	70		12	280		1	L290		
*		*	*		*		*	*		*		*	*	•	*
							GAT								
							CTA								
Cys	Val	Cys	His	Leu	Leu	Met	Asp	Asp	Val	Val	Ser	Ala	Asp	Asn	Tyr>
130	00		17	10		1	L320			133	2.0		4.5	340	
	*	*	***	*		*	*		*	10.	*	*	Δ.	*	
ACA	CTG	GAC	CTG	TGG	GCT	GGG	CAG	CAG	CTG	CTG	TGG	AAG	GGC	TCC	ጥጥር
							GTC								
															Phe>
	1250				- ^										
	1350		•	136	50		13	370		. :	L380			139	
*	*	<b>A</b> GC	*		*	*		*	CCC	*	*	220	*		*
* AAG	*			CAT	* GTG		CCC	* AGG		* CCA	* GGA			ACA	* GTT
* AAG TTC	* CCC GGG	TCG	CTC	CAT GTA	* GTG CAC	TTT	CCC GGG	* AGG TCC	CGG	* CCA GGT	* GGA CCT	TTG	GAC	ACA TGT	* GTT CAA
* AAG TTC	* CCC GGG	TCG	CTC	CAT GTA	* GTG CAC	TTT	CCC GGG	* AGG TCC	CGG	* CCA GGT	* GGA CCT	TTG	GAC	ACA TGT	* GTT
* AAG TTC	* CCC GGG Pro	TCG	CTC	CAT GTA His	* GTG CAC	TTT	CCC GGG	* AGG TCC	CGG Ala	* CCA GGT	* GGA CCT Gly	TTG	GAC	ACA TGT Thr	* GTT CAA
* AAG TTC Lys	* CCC GGG Pro	TCG Ser 400 *	CTC Glu	CAT GTA His	* GTG CAC Val 1410 *	TTT Lys	CCC GGG Pro	* AGG TCC Arg	CGG Ala 20	* CCA GGT Pro	* GGA CCT Gly	TTG Asn 430	GAC Leu	ACA TGT Thr	* GTT CAA Val> 1440
* AAG TTC Lys  * CAC	* CCC GGG Pro  1	TCG Ser 400 * AAT	CTC Glu GTC	CAT GTA His	* GTG CAC Val 1410 * GAC	TTT Lys ACT	CCC GGG Pro *	* AGG TCC Arg 14:	CGG Ala 20 * CTG	* CCA GGT Pro	GGA CCT Gly 1	TTG Asn 430 * AGC	GAC Leu AAC	ACA TGT Thr	GTT CAA Val> 1440 * TAT
* AAG TTC Lys  * CAC GTG	* CCC GGG Pro	TCG Ser 400 * AAT TTA	CTC Glu GTC CAG	CAT GTA His * TCC AGG	GTG CAC Val 1410 GAC CTG	TTT Lys ACT TGA	CCC GGG Pro * CTG GAC	AGG TCC Arg 142 CTG GAC	CGG Ala 20 * CTG GAC	* CCA GGT Pro  * ACC TGG	GGA CCT Gly 1 TGG ACC	TTG Asn 430 * AGC TCG	GAC Leu AAC TTG	ACA TGT Thr * CCG GGC	CAA Val> 1440 TAT ATA
* AAG TTC Lys  * CAC GTG	* CCC GGG Pro	TCG Ser 400 * AAT TTA	CTC Glu GTC CAG	CAT GTA His * TCC AGG	GTG CAC Val 1410 GAC CTG	TTT Lys ACT TGA	CCC GGG Pro * CTG GAC	AGG TCC Arg 142 CTG GAC	CGG Ala 20 * CTG GAC	* CCA GGT Pro  * ACC TGG	GGA CCT Gly 1 TGG ACC	TTG Asn 430 * AGC TCG	GAC Leu AAC TTG	ACA TGT Thr * CCG GGC	GTT CAA Val> 1440 * TAT
* AAG TTC Lys  * CAC GTG	* CCC GGG Pro	TCG Ser 400 * AAT TTA	GTC CAG Val	CAT GTA His * TCC AGG	GTG CAC Val 1410 * GAC CTG Asp	TTT Lys ACT TGA	CCC GGG Pro * CTG GAC	AGG TCC Arg 142 CTG GAC Leu	CGG Ala 20 * CTG GAC	CCA GGT Pro * ACC TGG	GGA CCT Gly 1 TGG ACC	TTG Asn 430 * AGC TCG	GAC Leu AAC TTG Asn	ACA TGT Thr * CCG GGC	CAA Val> 1440 * TAT ATA
* AAG TTC Lys  * CAC GTG	* CCC GGG Pro	TCG Ser 400 * AAT TTA Asn	GTC CAG Val	CAT GTA His * TCC AGG	GTG CAC Val 1410 * GAC CTG Asp	TTT Lys ACT TGA Thr	CCC GGG Pro * CTG GAC	AGG TCC Arg 142 CTG GAC Leu	CGG Ala 20 * CTG GAC Leu	CCA GGT Pro * ACC TGG	GGA CCT Gly 1 TGG ACC	TTG Asn 430 * AGC TCG Ser	GAC Leu AAC TTG Asn	ACA TGT Thr * CCG GGC	CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  CAC GTG His	CCC GGG Pro 1 ACC TGG Thr	TCG Ser 400 * AAT TTA Asn 14 GAC	GTC CAG Val 50 *	CAT GTA His * TCC AGG Ser	GTG CAC Val 1410 GAC CTG Asp	TTT Lys  ACT TGA Thr  460 * TAT	CCC GGG Pro * CTG GAC Leu	* AGG TCC Arg 14: CTG GAC Leu * CAT	CGG Ala 20 * CTG GAC Leu 1470 *	CCA GGT Pro * ACC TGG Thr	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14	GAC Leu AAC TTG Asn 80	ACA TGT Thr  * CCG GGC Pro	GTT CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  * CAC GTG His	CCC GGG Pro  1 ACC TGG Thr  * CCT GGA	TCG Ser 400 * AAT TTA Asn 14 GAC CTG	GTC CAG Val 50 * AAT	CAT GTA His * TCC AGG Ser TAC ATG	GTG CAC Val 1410 GAC CTG Asp	ACT TGA Thr 460 * TAT ATA	CCC GGG Pro * CTG GAC Leu	AGG TCC Arg 14: CTG GAC Leu * CAT GTA	CGG Ala 20 * CTG GAC Leu 1470 * CTC GAG	CCA GGT Pro * ACC TGG Thr	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14	AAC TTG Asn * GTC CAG	ACA TGT Thr  * CCG GGC Pro  * AAC	GTT CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  * CAC GTG His	CCC GGG Pro  1 ACC TGG Thr  * CCT GGA	TCG Ser 400 * AAT TTA Asn 14 GAC CTG	GTC CAG Val 50 * AAT	CAT GTA His * TCC AGG Ser TAC ATG	GTG CAC Val 1410 GAC CTG Asp	ACT TGA Thr 460 * TAT ATA	CCC GGG Pro * CTG GAC Leu	AGG TCC Arg 14: CTG GAC Leu * CAT GTA	CGG Ala 20 * CTG GAC Leu 1470 * CTC GAG	CCA GGT Pro * ACC TGG Thr	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14	AAC TTG Asn * GTC CAG	ACA TGT Thr  * CCG GGC Pro  * AAC	GTT CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  * CAC GTG His	CCC GGG Pro  1 ACC TGG Thr  * CCT GGA	TCG Ser 400 * AAT TTA Asn 14 GAC CTG Asp	GTC CAG Val 50 * AAT TTA Asn	CAT GTA His * TCC AGG Ser TAC ATG	GTG CAC Val 1410 GAC CTG Asp	ACT TGA Thr 460 * TAT ATA	CCC GGG Pro * CTG GAC Leu AAT TTA Asn	AGG TCC Arg 14: CTG GAC Leu * CAT GTA	CGG Ala 20 * CTG GAC Leu 1470 * CTC GAG Leu	* CCA GGT Pro  * ACC TGG Thr  ACC TGG	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14	AAC TTG Asn 80 * GTC CAG Val	ACA TGT Thr  * CCG GGC Pro  * AAC	GTT CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  * CAC GTG His  CCC GGG	CCC GGG Pro  1 ACC TGG Thr  * CCT GGA	TCG Ser 400 * AAT TTA Asn 14 GAC CTG Asp	GTC CAG Val 50 * AAT	CAT GTA His * TCC AGG Ser TAC ATG	GTG CAC Val 1410 GAC CTG Asp	ACT TGA Thr 460 * TAT ATA	CCC GGG Pro * CTG GAC Leu	AGG TCC Arg 14: CTG GAC Leu * CAT GTA	CGG Ala 20 * CTG GAC Leu 1470 * CTC GAG Leu	CCA GGT Pro * ACC TGG Thr	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14	AAC TTG Asn * GTC CAG	ACA TGT Thr  * CCG GGC Pro  * AAC	GTT CAA Val> 1440 * TAT ATA Tyr>
* AAG TTC Lys  * CAC GTG His  CCC GGG Pro	CCC GGG Pro 1 ACC TGG Thr * CCT GGA Pro	TCG Ser 400 * AAT TTA Asn 14 GAC CTG Asp	GTC CAG Val 50 * AAT TTA Asn 1500	CAT GTA His * TCC AGG Ser TAC ATG	GTG CAC Val 1410 * GAC CTG Asp CTG GAC Leu *	ACT TGA Thr 460 * TAT ATA Tyr	CCC GGG Pro * CTG GAC Leu AAT TTA Asn	* AGG TCC Arg 14: CTG GAC Leu * CAT GTA His	CGG Ala 20 * CTG GAC Leu 1470 * CTC GAG Leu	* CCA GGT Pro  * ACC TGG Thr  ACC TGG Thr  520 *	GGA CCT Gly 1 TGG ACC Trp	TTG Asn 430 * AGC TCG Ser 14 GCA CGT Ala	AAC TTG Asn 80 * CAG CAG Val	ACA TGT Thr  * CCG GGC Pro  * AAC	* GTT CAA Val> 1440  * TAT ATA Tyr> ATT TAA Ile>
* AAG TTC Lys  * CAC GTG His  CCC GGG Pro  1490  * TGG ACC	CCC GGG Pro  ACC TGG Thr  * CCT GGA Pro  AGT TCA	TCG Ser 400 * AAT TTA Asn 14 GAC CTG Asp	GTC CAG Val  AAT TTA Asn  1500  AAC TTG	CAT GTA His  * TCC AGG Ser  TAC ATG TYr	GTG CAC Val 1410 * GAC CTG Asp 1. CTG GAC Leu * CCG	ACT TGA Thr 460 * TAT ATA Tyr 15 GCA CGT	CCC GGG Pro  * CTG GAC Leu  AAT TTA Asn  10  * CAT	* AGG TCC Arg 14: CTG GAC Leu * CAT GTA His	CGG Ala 20 * CTG GAC Leu 1470 CTC GAG Leu 1 AGA	CCA GGT Pro * ACC TGG Thr ACC TGG Thr 520	GGA CCT Gly  1 TGG ACC Trp  * TAT ATA Tyr	TTG Asn 430 * AGC TCG Ser 14 GCA CGT Ala	AAC TTG Asn 80 CAG	ACA TGT Thr  * CCG GGC Pro  * AAC ASn	GTT CAA Val> 1440 * TAT ATA Tyr> ATT TAA Ile>

# Fig.32E.

1540 *		1550			1560				1570			1580			
CMA	*	*		*		*	*		*		*	*		*	
GAT	CTT	CCC	TCC	CTC	CGC	ATC	GCA	GCC	AGC	ACC	CTG	AAG	TCT	GGG	ATT
Leu	Glu	GGG Pro	Ser	Len	Ara	TAG	CGT	CGG λ1 a	TCG	TGG	GAC	TTC	AGA	CCC	TAA Ile>
			552		nr g	116	AIG	AIG	261	1111	Leu	гÀг	Ser	GIY	Ile>
	1590			16	00	1610			1620			1630			3 0
*	*		*		*	*		*		*	*		*		*
TCC	TAC	AGG	GCA	CGG	GTG	AGG	GCC	TGG	GCT	CAG	TGC	TAT	AAC	ACC	ACC
Ser	ጥሆተ	TCC	CGT	GCC	CAC	TCC	CGG	ACC	CGA	GTC	ACG	ATA	TTG	TGG	TGG
	-3-	•••	nıa	Arg	Val	Arg	Ala	TIP	ATA	GIN	Cys	Tyr	Asn	Thr	Thr>
	1640		1650			1660			50		16	570	570 16		
	* *			* *		*		*		*		*		1680 * *	
TGG	AGT	GAG	TGG	AGC	CCC	AGC	ACC	AAG	TGG	CAC	AAC	TCC	TAC	AGG	GAG
ACC	TCA	CTC	ACC	TCG	GGG	TCG	TGG	TTC	ACC	GTG	TTG	AGG	ATG	TCC	CTC
110	Ser	GIU	urp	Ser	Pro	Ser	Thr	Lys	Trp	His	Asn	Ser	Tyr	Arg	Glu>
		169	90		13	700		:	1710			172	2.0		
	*		*	*		*		*	*		*		*	*	
CCC	TTC	GAG	CAG	TCC	GGA	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA
GGG D×0	AAG	CTC	GTC	AGG	CCT	CTG	TTT	TGA	GTG	TGT	ACG	GGT	GGC	ACG	GGT
FIO	File	GIU	GIN	ser	GTA	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro>
			10				1760				1770				
1730		1	L740			175	50		11	760		1	770		
*		*	*		*		*	*		*		*	*		*
* GCA	ССТ	* GAA	* CTC	CTG	* GGG	GGA	* CCG	TCA	GTC	* TTC	CTC	* TTC	* CCC	CCA	AAA
* GCA CGT	GGA	* GAA CTT	* CTC GAG	GAC	CCC	GGA CCT	* CCG GGC	TCA AGT	GTC CAG	* TTC AAG	GAG	* TTC AAG	CCC GGG	GGT	AAA TTT
* GCA CGT	GGA	* GAA CTT	* CTC GAG	GAC	CCC	GGA CCT	* CCG GGC	TCA AGT	GTC CAG	* TTC AAG	GAG	* TTC AAG	CCC GGG	GGT	AAA
* GCA CGT	GGA Pro	* GAA CTT	* CTC GAG Leu	GAC	CCC	GGA CCT Gly	* CCG GGC Pro	TCA AGT	GTC CAG	* TTC AAG Phe	GAG Leu	* TTC AAG	* CCC GGG Pro	GGT Pro	AAA TTT
* GCA CGT Ala	GGA Pro 80	* GAA CTT Glu *	* CTC GAG Leu	GAC Leu '90 *	GJA	GGA CCT Gly	* CCG GGC Pro	TCA AGT Ser	GTC CAG Val	* TTC AAG Phe	GAG Leu L0	* TTC AAG Phe	CCC GGG Pro	GGT Pro	AAA TTT Lys>
GCA CGT Ala 17	GGA Pro 80 * AAG	* GAA CTT Glu * GAC	* CTC GAG Leu 17	GAC Leu '90 * CTC	CCC Gly ATG	GGA CCT Gly *	* CCG GGC Pro L800 * TCC	TCA AGT Ser	GTC CAG Val *	* TTC AAG Phe 181	GAG Leu LO *	* TTC AAG Phe * GTC	CCC GGG Pro	GGT Pro 20 *	AAA TTT Lys>
GCA CGT Ala 17 CCC GGG	GGA Pro 80 * AAG TTC	* GAA CTT Glu  * GAC CTG	CTC GAG Leu 17 ACC TGG	GAC Leu '90 * CTC GAG	CCC Gly ATG TAC	GGA CCT Gly * ATC TAG	CCG GGC Pro 1800 * TCC AGG	TCA AGT Ser CGG GCC	GTC CAG Val * ACC TGG	* TTC AAG Phe 181 CCT GGA	GAG Leu LO * GAG CTC	* TTC AAG Phe  * GTC CAG	CCC GGG Pro 18 ACA TGT	GGT Pro 20 * TGC ACG	AAA TTT Lys> GTG CAC
GCA CGT Ala 17 CCC GGG	GGA Pro 80 * AAG TTC	* GAA CTT Glu  * GAC CTG	CTC GAG Leu 17 ACC TGG	GAC Leu '90 * CTC GAG	CCC Gly ATG TAC	GGA CCT Gly * ATC TAG	CCG GGC Pro 1800 * TCC AGG	TCA AGT Ser CGG GCC	GTC CAG Val * ACC TGG	* TTC AAG Phe 181 CCT GGA	GAG Leu LO * GAG CTC	* TTC AAG Phe  * GTC CAG	CCC GGG Pro 18 ACA TGT	GGT Pro 20 * TGC ACG	AAA TTT Lys>
GCA CGT Ala 17 CCC GGG Pro	GGA Pro 80 * AAG TTC	GAA CTT Glu  * GAC CTG	CTC GAG Leu 17 ACC TGG	GAC Leu '90 * CTC GAG	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG	CCG GGC Pro 1800 * TCC AGG Ser	TCA AGT Ser CGG GCC	GTC CAG Val * ACC TGG	TTC AAG Phe 181 CCT GGA Pro	GAG Leu LO * GAG CTC	* TTC AAG Phe  * GTC CAG	CCC GGG Pro 18 ACA TGT	GGT Pro 320 * TGC ACG Cys	AAA TTT Lys> GTG CAC Val>
GCA CGT Ala  17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys	* GAA CTT Glu  * GAC CTG Asp	* CTC GAG Leu 17 ACC TGG Thr	GAC Leu '90 * CTC GAG Leu	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG Ile	CCG GGC Pro 1800 * TCC AGG Ser	TCA AGT Ser CGG GCC Arg	GTC CAG Val * ACC TGG Thr	TTC AAG Phe 181 CCT GGA Pro	GAG Leu 10 * GAG CTC Glu	* TTC AAG Phe  * GTC CAG Val	CCC GGG Pro 18 ACA TGT Thr	GGT Pro 320 * TGC ACG Cys	AAA TTT Lys> GTG CAC Val>
GCA CGT Ala  17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys 1830 *	GAA CTT Glu  * GAC CTG Asp	* CTC GAG Leu 17 ACC TGG Thr *	GAC Leu '90 * CTC GAG Leu 184	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG Ile	CCG GGC Pro 1800 * TCC AGG Ser	TCA AGT Ser CGG GCC Arg	GTC CAG Val * ACC TGG Thr	TTC AAG Phe 181 CCT GGA Pro 1	GAG Leu 10 * GAG CTC Glu 1860 *	* TTC AAG Phe  * GTC CAG Val	* CCC GGG Pro  18 ACA TGT Thr	GGT Pro 320 * TGC ACG Cys 187	AAA TTT Lys> GTG CAC Val>
GCA CGT Ala 17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys 1830 * GTG CAC	GAA CTT Glu  * GAC CTG Asp	CTC GAG Leu  17 ACC TGG Thr  * GTG CAC	GAC Leu '90 * CTC GAG Leu 184 AGC	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG Ile * GAA CTT	CCG GGC Pro 1800 * TCC AGG Ser 18	TCA AGT Ser CGG GCC Arg 50 * CCT GGA	GTC CAG Val * ACC TGG Thr	TTC AAG Phe  181 CCT GGA Pro  * GTC CAG	GAG Leu 10 * GAG CTC Glu 1860 * AAG	* TTC AAG Phe  * GTC CAG Val  TTC AAG	CCC GGG Pro 18 ACA TGT Thr	GGT Pro * TGC ACG Cys 187	AAA TTT Lys> GTG CAC Val> 0 * TAC ATG
GCA CGT Ala 17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys 1830 * GTG CAC	GAA CTT Glu  * GAC CTG Asp	CTC GAG Leu  17 ACC TGG Thr  * GTG CAC	GAC Leu '90 * CTC GAG Leu 184 AGC	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG Ile * GAA CTT	CCG GGC Pro 1800 * TCC AGG Ser 18	TCA AGT Ser CGG GCC Arg 50 * CCT GGA	GTC CAG Val * ACC TGG Thr	TTC AAG Phe  181 CCT GGA Pro  * GTC CAG	GAG Leu 10 * GAG CTC Glu 1860 * AAG	* TTC AAG Phe  * GTC CAG Val  TTC AAG	CCC GGG Pro 18 ACA TGT Thr	GGT Pro * TGC ACG Cys 187	AAA TTT Lys> GTG CAC Val>
GCA CGT Ala 17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys 1830 * GTG CAC Val	GAA CTT Glu  * GAC CTG Asp	CTC GAG Leu  17 ACC TGG Thr  * GTG CAC	GAC Leu '90 * CTC GAG Leu 184 AGC TCG Ser	CCC Gly ATG TAC Met	GGA CCT Gly * ATC TAG Ile * GAA CTT	CCG GGC Pro 1800 * TCC AGG Ser 18	TCA AGT Ser CGG GCC Arg 50 * CCT GGA	GTC CAG Val * ACC TGG Thr GAG CTC Glu	TTC AAG Phe  181 CCT GGA Pro  * GTC CAG	GAG Leu 10 * GAG CTC Glu 1860 * AAG TTC Lys	* TTC AAG Phe  * GTC CAG Val  TTC AAG Phe	CCC GGG Pro 18 ACA TGT Thr	GGT Pro * TGC ACG Cys 187 TGG ACC	AAA TTT Lys> GTG CAC Val> TAC ATG Tyr>
GCA CGT Ala 17 CCC GGG Pro	GGA Pro 80 * AAG TTC Lys 1830 * GTG CAC Val	GAC CTG Asp	* CTC GAG Leu 17 ACC TGG Thr  * GTG CAC Val	GAC Leu '90 * CTC GAG Leu 184 AGC TCG Ser	CCC Gly ATG TAC Met CAC GTG His	GGA CCT Gly * ATC TAG Ile * GAA CTT Glu	CCG GGC Pro 1800 * TCC AGG Ser 18 GAC CTG Asp	TCA AGT Ser  CGG GCC Arg  50 * CCT GGA Pro	GTC CAG Val * ACC TGG Thr GAG CTC Glu	TTC AAG Phe 181 CCT GGA Pro  * GTC CAG Val	GAG Leu GAG CTC Glu 4860 * AAG TTC Lys	* TTC AAG Phe  * GTC CAG Val  TTC AAG Phe	CCC GGG Pro 18 ACA TGT Thr * AAC TTG ASN	GGT Pro  20  * TGC ACG Cys  187  TGG ACC TTP	AAA TTT Lys> GTG CAC Val> TAC ATG Tyr>
GCA CGT Ala  17  CCC GGG Pro  * GTG CAC Val	GGA Pro 80 * AAG TTC Lys 1830 * GTG CAC Val	GAA CTT Glu  * GAC CTG Asp  GAC CTG Asp  380 * GGC	* CTC GAG Leu 17 ACC TGG Thr  * GTG CAC Val	GAC Leu '90 * CTC GAG Leu 184 AGC TCG Ser	CCC Gly ATG TAC Met CAC GTG His	GGA CCT Gly * ATC TAG Ile * GAA CTT Glu	CCG GGC Pro 1800 * TCC AGG Ser 18 GAC CTG Asp	TCA AGT Ser  CGG GCC Arg  50 * CCT GGA Pro  190 GCC	GTC CAG Val * ACC TGG Thr GAG CTC Glu	TTC AAG Phe 181 CCT GGA Pro 1 * GTC CAG Val	GAG Leu  GAG CTC Glu  AAG TTC Lys	* TTC AAG Phe  * GTC CAG Val  TTC AAG Phe	CCC GGG Pro  18  ACA TGT Thr  *  AAC TTG Asn	GGT Pro  220  * TGC ACG Cys  187  TGG ACC TTP	AAA TTT Lys> GTG CAC Val> TAC ATG Tyr>
* GCA CGT Ala 17 CCC GGG Pro  * GTG CAC Val	GGA Pro  80 * AAG TTC Lys 1830 * GTG CAC Val  18 GAC CTG	GAA CTT Glu  * GAC CTG Asp  GAC CTG Asp  880 * GGC CCG	* CTC GAG Leu 17 ACC TGG Thr  * GTG CAC Val	GAC Leu '90 * CTC GAG Leu 184 AGC TCG Ser	CCC Gly ATG TAC Met CAC GTG His .890 *	GGA CCT Gly * ATC TAG Ile * GAA CTT Glu	CCG GGC Pro  800 * TCC AGG Ser  18 GAC CTG Asp  * AAT TTA	TCA AGT Ser  CGG GCC Arg  50 * CCT GGA Pro 190 GCC CGG	GTC CAG Val * ACC TGG Thr GAG CTC Glu	TTC AAG Phe  181 CCT GGA Pro  * GTC CAG Val  * ACA TGT	GAG Leu  GAG CTC Glu  AAG TTC Lys  AAG TTC	* TTC AAG Phe  * GTC CAG Val  TTC AAG Phe  10 * CCG GGC	CCC GGG Pro  18 ACA TGT Thr  * AAC TTG ASn  CGG GCC	GGT Pro  220  * TGC ACG Cys  187  TGG ACC Trp	AAA TTT Lys> GTG CAC Val> TAC ATG Tyr>

# Fig.32F.

	1930					19	40		1950				1960				
		*		*	*		*		*	*		*		*	*		
	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC	CTC	ACC	GTC	CTG	CAC	
				TCG													
	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His>	
19	70		1	1980			199	0.0		20	00		-	2010			
	*		*	*		*	100	*	*	20	*		*	*		*	
-	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTC	TCC	AAC	AAA	
	GTC	CTG	ACC	GAC	TTA	CCG	TTC	CTC	ATG	TTC	ACG	TTC	CAG	AGG	TTG	TTT	
	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys>	
	2020		2030			2040			2050			50	20			160	
	*		*	* *		* *		*			* *			*			
				GCC													
				CGG													
	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln>	
	2	2070			208	30		20	90		2	2100			21:	10	
	*	*		*		*	*		*		*	*		* *		*	
				CCA													
				GGT													
	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met>	
2120			2130				2140										
		۷.	120		- 2	2130			214	40		2:	150		:	2160	
	*	4.	±		*	2130		*	214	40 *	*	2:	150 *		*	2160 *	
		AAG	* AAC	CAG	* GTC	* AGC			TGC	* CTG		AAA	* GGC		* TAT	* CCC	
	TGG	AAG TTC	* AAC TTG	GTC	* GTC CAG	* AGC TCG	GAC	TGG	TGC ACG	* CTG GAC	CAG	AAA TTT	* GGC CCG	AAG	* TAT ATA	* CCC GGG	
	TGG	AAG TTC	* AAC TTG	GTC	* GTC CAG	* AGC TCG	GAC	TGG	TGC ACG	* CTG GAC	CAG	AAA TTT	* GGC CCG	AAG	* TAT ATA	* CCC	
	TGG	AAG TTC	* AAC TTG	GTC Gln	* GTC CAG	* AGC TCG Ser	GAC	TGG	TGC ACG Cys	* CTG GAC	CAG	AAA TTT	* GGC CCG	AAG Phe	* TAT ATA	* CCC GGG	
	TGG Thr	AAG TTC Lys	* AAC TTG Asn 21	GTC Gln 70	* GTC CAG Val	* AGC TCG Ser	GAC Leu 180	TGG Thr	TGC ACG Cys	* CTG GAC Leu 2190 *	CAG Val	AAA TTT Lys	* GGC CCG Gly	AAG Phe 00	* TAT ATA Tyr	* CCC GGG Pro>	
	TGG Thr	AAG TTC Lys * GAC	* AAC TTG Asn 21	GTC Gln 70 * GCC	* GTC CAG Val  * GTG	AGC TCG Ser 2:	GAC Leu 180 * TGG	TGG Thr GAG	TGC ACG Cys *	* CTG GAC Leu 2190 * AAT	CAG Val	AAA TTT Lys *	* GGC CCG Gly 22 CCG	AAG Phe 00 * GAG	* TAT ATA Tyr  * AAC	* CCC GGG Pro>	
	TGG Thr AGC TCG	AAG TTC Lys * GAC CTG	* AAC TTG Asn 21' ATC TAG	GTC Gln 70 * GCC CGG	* GTC CAG Val  * GTG CAC	AGC TCG Ser 2: GAG CTC	GAC Leu 180 * TGG ACC	TGG Thr GAG CTC	TGC ACG Cys * AGC TCG	CTG GAC Leu 2190 AAT TTA	CAG Val GGG CCC	AAA TTT Lys * CAG GTC	* GGC CCG Gly 22 CCG GGC	AAG Phe 00 * GAG CTC	TAT ATA Tyr  * AAC TTG	* CCC GGG Pro>	
	TGG Thr AGC TCG	AAG TTC Lys * GAC CTG	* AAC TTG Asn 21' ATC TAG	GTC Gln 70 * GCC CGG	* GTC CAG Val  * GTG CAC	AGC TCG Ser 2: GAG CTC	GAC Leu 180 * TGG ACC	TGG Thr GAG CTC	TGC ACG Cys * AGC TCG	CTG GAC Leu 2190 AAT TTA	CAG Val GGG CCC	AAA TTT Lys * CAG GTC	* GGC CCG Gly 22 CCG GGC	AAG Phe 00 * GAG CTC	TAT ATA Tyr  * AAC TTG	* CCC GGG Pro>	
2:	TGG Thr AGC TCG	AAG TTC Lys * GAC CTG	* AAC TTG Asn 21' ATC TAG Ile	GTC Gln 70 * GCC CGG	GTC CAG Val	AGC TCG Ser 2: GAG CTC	GAC Leu 180 * TGG ACC	TGG Thr GAG CTC Glu	TGC ACG Cys * AGC TCG	CTG GAC Leu 2190 * AAT TTA Asn	CAG Val GGG CCC	AAA TTT Lys * CAG GTC	GGC CCG Gly 22 CCG GGC Pro	AAG Phe 00 * GAG CTC	TAT ATA Tyr  * AAC TTG Asn	* CCC GGG Pro>	
22	TGG Thr AGC TCG Ser	AAG TTC Lys * GAC CTG	* AAC TTG Asn 21' ATC TAG Ile	GTC Gln 70 * GCC CGG Ala	* GTC CAG Val  * GTG CAC Val	AGC TCG Ser 2: GAG CTC	GAC Leu 180 * TGG ACC	TGG Thr GAG CTC Glu	TGC ACG Cys * AGC TCG	CTG GAC Leu 2190 * AAT TTA Asn	CAG Val GGG CCC Gly	AAA TTT Lys * CAG GTC	GGC CCG Gly 22 CCG GGC Pro	AAG Phe 00 * GAG CTC Glu	* TAT ATA Tyr  * AAC TTG Asn	CCC GGG Pro>	
22	TGG Thr AGC TCG Ser 210 *	AAG TTC Lys * GAC CTG Asp	* AAC TTG Asn 21' ATC TAG Ile * ACC	GTC Gln 70 * GCC CGG Ala 2220 *	GTC CAG Val	* AGC TCG Ser 2: GAG CTC Glu * CCC	GAC Leu 180 * TGG ACC Trp 22:	TGG Thr  GAG CTC Glu  30 * CTG	TGC ACG Cys * AGC TCG Ser	CTG GAC Leu 2190 * AAT TTA Asn 22	GGG CCC Gly 240	AAA TTT Lys * CAG GTC Gln	* GGC CCG Gly 22 CCG GGC Pro	AAG Phe  OO * GAG CTC Glu 2250 *	* TAT ATA TYr  * AAC TTG Asn	* CCC GGG Pro>  AAC TTG Asn>  * CTC	
2:	TGG Thr AGC TCG Ser 210 * TAC ATG	AAG TTC Lys  * GAC CTG Asp	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG	GTC Gln 70 * GCC CGG Ala 2220 * ACG	GTC CAG Val	AGC TCG Ser 2: GAG CTC Glu  * CCC GGG	GAC Leu 180 * TGG ACC Trp 22: GTG CAC	TGG Thr  GAG CTC Glu  CTG GAC	TGC ACG Cys * AGC TCG Ser * GAC CTG	CTG GAC Leu 2190 * AAT TTA Asn CC AGG	GGG CCC Gly 240 * GAC CTG	AAA TTT Lys CAG GTC Gln	* GGC CCG Gly 22 CCG GGC Pro * TCC AGG	AAG Phe  00 * GAG CTC Glu 2250 * TTC AAG	* TAT ATA TYr  * AAC TTG Asn  TTC AAG	CCC GGG Pro>  AAC TTG Asn>  CTC GAG	
2:	TGG Thr AGC TCG Ser 210 * TAC ATG	AAG TTC Lys  * GAC CTG Asp	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG	GTC Gln 70 * GCC CGG Ala 2220 * ACG	GTC CAG Val	AGC TCG Ser 2: GAG CTC Glu  * CCC GGG	GAC Leu 180 * TGG ACC Trp 22: GTG CAC	TGG Thr  GAG CTC Glu  CTG GAC	TGC ACG Cys * AGC TCG Ser * GAC CTG	CTG GAC Leu 2190 * AAT TTA Asn CC AGG	GGG CCC Gly 240 * GAC CTG	AAA TTT Lys CAG GTC Gln	* GGC CCG Gly 22 CCG GGC Pro * TCC AGG	AAG Phe  00 * GAG CTC Glu 2250 * TTC AAG	* TAT ATA TYr  * AAC TTG Asn  TTC AAG	* CCC GGG Pro>  AAC TTG Asn>  * CTC	
22	TGG Thr AGC TCG Ser 210 * TAC ATG	AAG TTC Lys  * GAC CTG Asp  AAG TTC Lys	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG	GTC Gln 70 * GCC CGG Ala 2220 * ACG TGC	GTC CAG Val	AGC TCG Ser 2: GAG CTC Glu  * CCC GGG	GAC Leu 180 * TGG ACC Trp 22: GTG CAC Val	TGG Thr  GAG CTC Glu  CTG GAC	TGC ACG Cys * AGC TCG Ser * GAC CTG	CTG GAC Leu 2190 * AAT TTA Asn CC AGG	GGG CCC Gly 240 * GAC CTG	AAA TTT Lys * CAG GTC Gln GGC CCG Gly	* GGC CCG Gly 22 CCG GGC Pro * TCC AGG	AAG Phe  00 * GAG CTC Glu 2250 * TTC AAG	* TAT ATA TYr  * AAC TTG Asn  TTC AAG	CCC GGG Pro>  AAC TTG Asn>  CTC GAG	
2:	AGC TCG Ser TAC ATG Tyr 22	AAG TTC Lys  * GAC CTG Asp  AAG TTC Lys	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG Thr	GTC Gln 70 * GCC CGG Ala 2220 * ACG TGC Thr	* GTC CAG Val CCT GGA Pro	* AGC TCG Ser 2: GAG CTC Glu * CCC GGG Pro	GAC Leu 180 * TGG ACC Trp 22: GTG CAC Val	GAG CTC Glu 30 * CTG GAC Leu 2280	TGC ACG Cys * AGC TCG Ser GAC CTG Asp	CTG GAC Leu 2190 * AAT TTA Asn CC AGG Ser	GGG CCC Gly ASP 22	AAA TTT Lys CAG GTC Gln GGC CCG Gly	* GGC CCG Gly 22 CCG GGC Pro * TCC AGG Ser	AAG Phe  00 * GAG CTC Glu  2250 * TTC AAG Phe	* TAT ATA TYr  * AAC TTG Asn  TTC AAG Phe 300 *	* CCC GGG Pro>  AAC TTG Asn>  * CTC GAG Leu>	
22	TGG Thr  AGC TCG Ser  10 * TAC ATG Tyr 22	AAG TTC Lys  * GAC CTG Asp  AAG TTC Lys  60 * AGC	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG Thr	GTC Gln 70 * GCC CGG Ala 2220 * ACG TGC Thr	* GTC CAG Val CCT GGA Pro	AGC TCG Ser 2: GAG CTC Glu  * CCC GGG Pro	GAC Leu 180 * TGG ACC Trp 22: GTG CAC Val	GAG CTC Glu CTG GAC Leu AAG	TGC ACG Cys  * AGC TCG Ser  GAC CTG Asp	CTG GAC Leu 2190 * AAT TTA Asn TCC AGG Ser *	GGG CCC Gly ASP 22	AAA TTT Lys CAG GTC GIn GGC CCG Gly 90 *	* GGC CCG Gly 22 CCG GGC Pro  * TCC AGG Ser	AAG Phe  00 * GAG CTC Glu  2250 * TTC AAG Phe	* TAT ATA TYT  * AAC TTG AAG Phe 300 *	* CCC GGG Pro>  AAC TTG Asn>  * CTC GAG Leu>	
22	TGG Thr AGC TCG Ser TAC ATG Tyr 22 TAT ATA	AAG TTC Lys  * GAC CTG Asp  AAG TTC Lys  60 * AGC TCG	* AAC TTG Asn 21' ATC TAG Ile * ACC TGG Thr AAG TTC	GTC Gln 70 * GCC CGG Ala 2220 * ACG TGC Thr	* GTC CAG Val CCT GGA Pro ACC TGG	* AGC TCG Ser 2: GAG CTC Glu * CCC GGG Pro	GAC Leu 180 * TGG ACC Trp 22: GTG CAC Val * GAC CTG	GAG CTC Glu CTG GAC Leu 2280 *	TGC ACG Cys  * AGC TCG Ser  * GAC CTG Asp	CTG GAC Leu 2190 * AAT TTA Asn  TCC AGG Ser * AGG TCC	GGG CCC Gly ASP 22 TGG ACC	AAA TTT Lys  CAG GTC GIn  GGC CCG Gly  CAG GTC	* GGC CCG Gly 22 CCG GGC Pro  * TCC AGG Ser  * CAG	AAG Phe  00 * GAG CTC Glu 2250 * TTC AAG Phe  2	* TAT ATA TYr  * AAC TTG Asn  TTC AAG Phe 300 * AAC	* CCC GGG Pro>  AAC TTG Asn>  * CTC GAG Leu>	

Fig.32G.

